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PLANT PHYSIOLOGY

OCTOBER, 1946

THE CASE FOR GREATER COOPERATION AMONG THE PLANT SCIENCE SOCIETIES[1]

BERNARD S. MEYER

Dispersive tendencies have long been at work among the biological organizations in this country, with the result that there are today at least thirty-five different biological societies of national scope. The aggregate membership of these organizations is between 30,000 and 40,000. Yet with only two or three exceptions, none of these societies has a membership of more than 2,000, and most of them are considerably smaller. Each of these organizations has its president, vice-president, secretary, and other officers. Many of them publish their own journals. With only one or two exceptions, all of these societies are run on a strictly amateur basis, the often arduous duties of secretaries, treasurers, editors, and business managers being performed gratuitously by those members who have been elected or appointed to such positions by the combined efforts of their friends and enemies.

The organizational division and subdivision of the biological fraternity is a matter of sincere concern to many thoughtful plant and animal scientists today. In many ways the lack of centralized organizations of biologists has operated against them, and will continue to do so. At no time has this fundamental weakness in the body politic of biologists become more glaringly apparent than during the war. The lack of a coherent and integrated focal organization contributed to the comparatively ineffective ultilization of the specialized and often unique talents of biological scientists in the war efforts.

Appalling examples of the misuse of biological manpower during the war can be cited. One of the most brilliant of the younger plant cytogeneticists in this country died on the beaches of Salerno. Another promising young botanist perished in the torpedoing of a Japanese prison ship off Mindanao. And these are not the only biologists whose contributions have been lost forever. Such men as these should never have been in the army at all. And of all branches of the army least of all should they have been in the infantry. Not even by their own choice should they have been allowed in the forefront of the fighting, not for their own sake, but for the sake of

[1] Delivered as the address of the retiring president of the American Society of Plant Physiologists for 1943–44 at St. Louis, Missouri, March 29, 1946.

the national welfare. There were dozens of more important jobs for them, both in and out of the armed services.

Less tragic, but still serious when measured in the currency of social loss, because of the multiplicity of cases, was the waste of biological manpower by the armed services under the guise of making proper use of it. Many a capable botanist or zoologist was assigned to the medical corps, for example, only to find himself relegated to some trivial non-biological occupation, or to the performance of some routine biological test at about the technological level of a sophomore in pre-medical school.

Nor was the misuse, or failure to use biological manpower where needed, confined to the armed forces. The situation in civilian life was not notably better. Many new and unexpected research problems arose out of the necessities and exigencies of global war. Many of these problems were either biological or they had important biological aspects. But biological manpower was never effectively mobilized for work on such problems. Sometimes the right man fell into the right job, but the contrary was more often true. Biologists were overlooked, bypassed, or given only secondary responsibilities in scientific endeavors for which they were fully competent to assume leadership.

One glaring example of the failure to direct botanical manpower into the proper channels was described by Dr. Griggs in his address at the Cleveland meeting several years ago. Over half a million dollars were reportedly spent on a certain fungous project which could have been done by competent mycologists for a small fraction of the sum. The agency in charge did not know where to turn for assistance; indeed, there is no evidence that they were aware of the existence of mycologists.

Some miscasting of actors is inevitable in the hastily plotted drama of total war. But the non-use or misuse of biological manpower was so widespread, so glaring, and at such a variance with sound public policy, that it should be a matter of serious concern to all plant and animal scientists.

No one person nor group is at fault for these costly and tragic mistakes in the utilization of scientific manpower. Some of the fault lies near home. Some of it even lies within this room, with you, with me, and with all other biologists. We failed ourselves and our colleagues when we neglected to provide a mechanism by which the possible services of biologists could be brought into better focus in the minds of governmental agencies, both military and civilian. If one or two strong, centralized bodies of biologists had been in existence, and had properly exerted their influence, it is certain that fewer mistakes in the allocation of biological manpower would have been made.

The war has now receded into history, but the duration is proving to be much longer than the war. We are now in a difficult period of postwar readjustment which is a part of the world-wide political, economic, and scientific disequilibrium. We—as plant scientists—were not properly organized for war, but it is not too late to organize for peace. Other problems

in which plant and animal scientists have a legitimate interest and about which they will wish to speak will constantly be coming to the fore. Without spokesmen, however, whose voices can ring with the authoritative support of all biologists, or at least of substantial groups of biologists, the scientists' chances of being heard will be very slim.

The bill for the establishment of a National Science Foundation now emerging from a congressional committee represents the most outstanding current project in which scientists have a vital stake. Polls indicate that an overwhelming majority of scientists is in favor of such legislation. After a somewhat disconcerting period of diversity of opinion and controversy, the sentiment of most scientists seems finally to be crystallizing behind S 1850, which embodies most of the best features of several previous bills.

The writing of a bill is only the first step in legislation and by no means insures its passage. No version of a bill for a National Science Foundation has ever been considered on the floor of the Senate, and the House is scarcely aware of the existence of such proposed legislation.[2] Neither is there any great public clamor in support of such a bill. A rising sentiment for economy may also prejudice Congress against new ways of spending money. Any hope for the passage of such legislation may therefore yet depend on the mobilization of the maximum possible influence of scientists themselves in its support. Strongly centralized bodies of scientists (organizations conspicuously lacking among biologists), are necessary if the most effective representations in support of the passage of such legislation are to be made.

If such a bill is passed, and a National Science Foundation becomes a reality, there will be the problem of continuing relations with this organization. The necessary liaison between individual biologists or small groups of biologists in specialized fields and such a national foundation could best be accomplished through the intermediary of a centralized coordinating organization.

Of all the major groups of scientists, none has lagged so far behind in the development of organizational coherence and esprit de corps as the biologists. The chemists are strongly entrenched in the bosom of their powerful American Chemical Society. The physicists have their American Institute of Physics. The psychologists have recently stifled secessionist movements within their ranks by the formation of a new centralizing organization. The constitution for an American Geological Institute has recently been drawn up by representatives of eleven geological societies, and in all probability such an Institute will soon exist in reality as well as on paper.

Attemps to bring the biological sciences into closer organizational communion have not been entirely lacking. The Union of American Biological Societies, designed to accomplish this aim, was organized in 1923. Although the originally expressed purposes of the association were much broader, in

[2] Bill S 1850 was subsequently passed by the Senate, but the House committee to which it was referred failed to report it out. This, in effect, killed all such legislation for the recent session of Congress.

actual operation its activity has been largely confined to the sponsorship of the journal, *Biological Abstracts*. Membership of this organization is by societies, thirty-two of which now belong to the Union. The American Biological Society, whose objectives in general are similar to those of the Union of American Biological Societies, is a comparative newcomer to the biology landscape, having been started in 1941. Membership in the American Biological Society is by individuals, however, and not by societies. A move is now on foot to merge these two societies into a single stronger and more effective organization.

There are many proponents of some sort of a plan for bringing all species of biologists, either directly or indirectly, into one large "supersociety." Some move in this direction seems not only desirable, but almost imperative. Theoretically, this might be accomplished in several ways: one would be to expand some existing society into a position of leadership. A second would be to enlist every individual biologist into a new society which would be organized for the explicit purpose of representing the common interests of biologists. A third would be to form a federation of existing societies implemented with a central coordinating board or council and a full-time executive officer who could adequately and aggressively represent all the life sciences.

For various reasons the last-mentioned plan for a league of biological societies seems the most feasible one for strengthening the professional position of biologists. The framework of such a federation is already in existence in the Union of American Biological Societies. In the past the organization of this society has been too loose, and its existence too remote from the consciousness of individual plant and animal scientists for it to serve as an effective coordinating agency for biologists. But with a strengthening of its joints, a re-tooling of its objectives, and centralization of its efforts, the Union might conceivably be converted into an agency suitable to represent and to command respect for all of biology.

The proposal to unite all biologists under a single banner has definite merits and advantages. One of the most obvious advantages is the mobilization of the greatest possible numerical strength behind a central organization which would result. There are also difficulties and disadvantages to any project attempting to include scientists of every biological denomination. One difficulty is to persuade a sufficient number of biological societies to subscribe to an effective plan for cooperation. There are more than thirty such societies, and biologists, individually and collectively, are notorious individualists. Most biological societies have, in the past, maintained their affiliation with the Union of American Biological Societies, but it must be remembered that they have been asked for little more than moral support. Because an effective union must make heavy demands of time, money, and energy, the reluctance of individual societies to join or to maintain membership may rise steeply.

Another difficulty is the great diversity of interests that would be en-

compassed by such an organization. A union of all biological societies embraces a diversity of interest, although not an aggregate membership, so great as that represented by all of chemistry, all of physics, plus a sizeable segment of the engineering sciences. The greater the number of societies which affiliate through any sort of a central organization, the greater the diversity of interests represented. The diversity may prove an internal source of weakness and friction and may make adequate representation of all interests difficult. In particular, it seems that plant scientists might legitimately raise the question of whether their welfare could be best served by incorporation into any kind of an association in which they will be greatly outnumbered by animal scientists.

I am not attempting to discourage or disparage any efforts to federate all biological societies into a strong league for their mutual benefit, but I am attempting to look at the problem realistically. Certainly, such a plan is one possible solution to the problem under discussion, and it may be the best one. But in view of certain disadvantages of the project for a comprehensive biological union, and also in view of the fact that plans for an effective organization of this kind are in the embryonic stage, certain alternative proposals should perhaps also be given consideration.

Although certain great unifying principles run through all of biology, it does not necessarily follow that biology is *de facto* to be considered a unit science. Similar great principles run through all of physics and chemistry, though these two sciences are invariably recognized as separate and autonomous fields of inquiry. The term "biology" is taxonomically more nearly on a par with the term "physical sciences" than with the narrower designations "physics" and "chemistry."

There is a nautral line of cleavage between the plant sciences and the animal sciences. Latterly, it has become the fashion in some circles to ignore the obvious dichotomy of interest, or to pretend that it does not exist. The demarkation between these two groups of biological sciences, however, is of the same order of magnitude as that between physics and chemistry, and to overlook it is to be blind to the facts of life. Of course there are overlapping areas, most of which represent important fields of scientific inquiry, but the same is true, and to relatively the same degree, of physics and chemistry.

An alternative suggestion would therefore seem to be that a Union of Plant Science Societies might be a more appropriate standard around which plant scientists could rally, than a federation of all biological societies. Organization of such a union would not necessarily exclude subsequent amalgamations with other biologists. There are only about ten national societies devoted exclusively to plant science, and I include in this group those in the applied fields such as horticulture, agronomy and forestry, as well as the more narrowly botanical groups. The chance of persuading such a group of societies to cooperate effectively through some sort of a superorganization appears more hopeful than the chance of inducing the thirty-

odd biological societies to fall into step with some similar proposal. The attainment of greater cooperation between the applied and theoretical plant sciences, which should be a by-product of such a cooperative project, would in itself be a worthy objective.

No plan should be followed for either a union of plant science societies or a union of all biological societies which will disturb the structural organization of existing societies. Most of the present societies have not only a long and honorable tradition, but also the established loyalties of their members. Any scheme which would attempt to disrupt these elements of the *status quo* would justifiably meet vigorous opposition.

The history of the American Institute of Physics is a pertinent object lesson for all botanists and zoologists. In 1931 the physicists of this country found themselves in a situation similar to that in which the biologists find themselves today. At that time the five major physics societies banded together to organize the American Institute of Physics. The organization has since acted as the spearhead for many kinds of cooperative activities among physicists. Membership in this Institute is by societies, not by individuals, but the member societies do not relinquish to the Institute their autonomy, title to their journals, control of meetings, maintenance of membership standards, or the responsibilities of their officers. The Institute is managed by a governing board to which three members are elected from each of the five constituent societies.

The activities of this organization have been manifold and only a few of the more important can be mentioned. The Institute assumed an important advisory rôle regarding the relation of physics and physicists to the war effort. It conducts a placement service for physicists. It considers questions of the establishment and maintenance of professional standards. Through the more dignified channels of publicity, it seeks to call attention to the achievements of physics. It cultivates closer and more understanding relations between physics and industry.

One of the major activities of the Institute has been supervising the publication of journals for the member societies, as well as publishing several journals in its own right. Major savings in publication costs have been achieved. Under this form of administration the constituent societies have paid out of dues, over a nine-year period, only 11 per cent. of the cost of publishing their journals. In addition, editors of the various journals have been relieved of many of the routine problems of publication.

Establishment of the Institute has assisted the member societies to augment their membership lists, increase the circulation of their journals, and advance their prestige in academic and industrial circles.

The Institute draws its financial support from a relatively small annual allowance from each of the member societies, profits of its journals and advertising therein, contributions of industrial and educational organizations, and grants from foundations.

Whether or not one is thinking of a plant science institute, an animal

science institute, or a federation of all biological societies, the American Institute of Physics provides a suggestive blueprint. Advantages and methods of financing such as those which the physicists have obtained might reasonably be expected to accrue to any sizeable group of biological societies pooling their common interests along lines somewhat similar to the American Institute of Physics.

Time permits only a sketchy presentation of the entire topic. But perhaps some of the verbal seed I have tried to sow will fall on receptive mental soils. The time for action in this matter will never be riper than the present. Only two courses are open: one is for us as individuals and as an organization to strive for a more centralized representation of our own and related sciences. The other, and unfortunately easier, course is to fall into a do-nothing or even a defeatist attitude. Each of us may say, "I am only one man. What can I do?" Or we may say, "This is only a small society. What can it accomplish?" Or we may even say, "Suppose we did have some sort of a union of biological societies or plant science societies, what could it do? The constituency of even such a group would be relatively so small in proportion to the enormous population of the country—could it really make its influence felt?"

Such sentiments remind me of an episode of my undergraduate days. There used to be, at Ohio State University, an annual fracas called the "cane rush," which took place every autumn between the freshmen and sophomores. The usually large horde of freshmen lined up at one end of a football field, and a much smaller group of sophomores at the other. The object was to carry a cane, initially in the sophomores' possession, to the opponents' goal posts. At a signal each group charged from its end of the field toward its opponents. The freshmen, by sheer weight of numbers, invariably won, despite any deception or artifice practiced by the sophomores. The inevitable outcome was not surprising as there were usually four or five freshmen to dispose of each sophomore. One year, however, the result was different. When the teams lined up, the sophomores did not assume their customary dispersed formation, but instead, arranged themselves into a wedge-shaped phalanx. Each man in the outermost row had his arms firmly linked with those of each of the men next to him. Within the outer row were other rows of men similarly linked together. Somewhere within this living wedge was the cane. Slowly but irresistibly the massive human triangle crawled down the field, clawed at by a surging horde of freshmen. But all attempts to dismember the phalanx failed. Some freshmen even took flying leaps on top of the phalanx—and promptly disappeared. Occasionally a man was peeled off the outer row of sophomores, but the gaps were always closed. When the final gun sounded the sophomores were across the freshman goal line with the cane still in their possession.

DEPARTMENT OF BOTANY
OHIO STATE UNIVERSITY
COLUMBUS 10, OHIO

THE COMING OF AGE OF THE AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS[1]

H. A. Spoehr

March 25, 1924, was the official birthday of our society. Owing to the exigencies of the war we were prevented from observing the attainment of its majority. As I had the honor of being president of the society during the twenty-first year of its existence, I feel that it may not be out of place to cast a retrospective glance over the youthful years of our society's life and to examine briefly what may lie ahead in its years of manhood. In venturing upon this theme let me stress that this is in no sense an official historical account; the opinions to be expressed are based upon my own impressions and should not be construed as representing a policy, or trend in policy, of the American Society of Plant Physiologists.

The birth of our society was not accomplished without some rather sharp birth pains. Yet, it is a kind provision of Nature that the throes of birth are soon forgotten in the joy of witnessing the young hopeful's growth and in the anxiety for its welfare. It has been a real satisfaction for all of us to have had a part in this experience. Sharing in this development has done much to foster the feeling of parental pride and cooperation which is characteristic of this group. A little more than a year after its birth we had the thrill of seeing our youngster cut his first tooth in the form of Volume I, Number 1, of our journal, PLANT PHYSIOLOGY. Since then we have had the gratification of seeing this journal attain its place among the foremost scientific publications of the world and become our most prized possession.

The fine things in this world are usually accomplished by men who do more than their share. This is eminently true of those who had the responsibility of editing our journal, particularly Charles A. Shull and the succession of secretary-treasurers, to whom the destiny of our hopeful venture was largely entrusted. It is but fitting that at this juncture in the life of our society we express to these loyal guardians our continued gratitude and devotion.

Our membership has shown a gratifying and wholesome growth. The first list of members, published May 10, 1924, carried 76 names. Today we have 623 members. There are some advantages in a relatively small society for the purposes to which we are devoted. The intimacy, fraternal feeling, and spirit of cooperation engendered by a group in which the members are well acquainted have many advantages. We still have some missionary work to do so that those plant physiologists outside of our ranks and scientists in related fields may also enjoy the advantages which are ours through membership in the society. In this connection it may be advisable for the society to give consideration to enlisting the interest and support of in-

[1] Retiring President's address, Saint Louis, Missouri, March 29, 1946.

dustries dealing with plant products through issuing corporate memberships in our society. The experience of other scientific bodies has demonstrated the mutual benefits which can result from such an arrangement. The attainment of our majority should not mean termination of our growth. Each one of us represents a cell in the organism of the society; the development of this organism and the direction in which it is to grow in large measure depend upon the functioning of each individual cell.

Financially we are in a sound, though not affluent condition. Each year we have laid down a small annual ring to strengthen the total structure. The current inflation and low interest rates have been a source of much concern to the finance committee and to the business management. Every effort is being made to conserve our resources consistent with conservative administration, and especially to preserve the quality and usefulness of our journal. The devastation caused by the war places new and heavy responsibilities on the scientific institutions of this country. In this connection our society and our journal, in spite of their youth, must bear their share.

It has been the custom for untold ages on the occasion of a young man's coming of age, that his elders feel it their duty and privilege to give him sound and sober advice as to his conduct in manhood. I hardly feel equal to this rôle in the present instance. But it seems to me that this period of our life as a society offers opportunity for a little self-examination and study of what we are to make of ourselves and what we have to contribute to the world in which we are to live. More compelling for such an undertaking is the indisputable fact that in no epoch of human history has mankind been faced with such serious problems as confront us today. There is, moreover, the further fact that, directly and indirectly, many of these problems and conditions are the result of scientific discovery and endeavor. Mankind is looking to scientists to do their share in the huge task of solving the innumerable problems entailed in the establishment of a peaceful world. As a fundamental scientific discipline, plant physiology has some very real responsibilities in this connection. Our science had little to do with the destructive aspects of the war; it may have all the greater opportunity as a reconstructive influence for peace and good will.

It would be presumptuous in this gathering to try to define the objectives of plant physiology or to recount its accomplishments. As one scans the development of our science during the past century or so, with all due modesty, one is impressed with the large number of important fundamental discoveries which have emanated from plant physiological laboratories. Thus, osmosis, the composition of proteins, chromatographic analysis, to mention but a few, had their inception as a result of plant physiological investigation. And as one examines more carefully the foundations of our scientific structure one finds that there is hardly any branch of biological science which has not been fed with valuable raw material from plant physiology. In fact, being among friends, it may be said that plant physiology is at the center of plant biology. Time and again plant physiology has produced

buds which, when grafted to other disciplines, produced fruitful new branches on the tree of knowledge. In this manner important aspects of plant physiology have been grafted to horticulture, biochemistry, nutrition, medicine, and many other sciences. In the rapid development of modern science little attention is paid to origins; it is the fully matured fruit which commands attention on the market place, not the bud from which it sprang. Thus, while plant physiology can be justly proud of its contributions, these are all too frequently recognized only by the few comprising our select circle. It has been truly said that plant physiology is the "Cinderella of Botany."

Tempting as an analysis of this situation is, to penetrate a little more thoroughly into the underlying causes and influences, such a digression would take us far afield and beyond the memory of our young celebrant. As a phenomenon in the growth of a scientific discipline and for the purpose of clarifying the interrelationships of the various aspects of plant biology, a more thorough study of the history of plant physiology may be very rewarding. It would be quite appropriate for a committee of our society to begin its study of the place of plant physiology in biological research and in education with a consideration of its historical development in relation to other sciences.

Without presuming to have given this problem the study and thought it deserves, one aspect thereof is rather clearly revealed through a perusal of the contributions which have appeared in our journal since its founding and in other similar organs. During the past half century or so, at least since plant physiology became primarily an experimental science through the impacts of physics and chemistry, the researches in plant physiology have been primarily of an observational nature. That is, efforts were made to advance knowledge through direct, immediate discriminations of particular observations. This has been, of course, sound procedure. Experimentation with living things is vastly more complex and difficult than experimentation with inanimate systems. It was necessary to accumulate a great deal of experience in order to build a sound foundation for the structure of the science. There was so much to do, there were so many different phenomena and such a variety of organisms, that observation on the basis of experimentation was the order of the day and the on-coming generations of plant physiologists breathed almost exclusively the atmosphere of observation.

Without attempting to become too philosophical, it is nevertheless a matter of common experience that what we call science grows by the interaction of two orders of experience. These are observation and interpretation. The observations must be interpreted in terms of concepts. Neither can be said to be more valuable than the other for the creation of a real science. The one may modify the other, but what leads to further investigation and what we retain is usually in the form of concepts. It is the way of our conceiving of Nature.

Although there were some outstanding exceptions, it would appear that

plant physiology has been long on observation and a little deficient in the conceptual aspects. Through observation plant physiology has made a number of outstanding discoveries. It has all too often left the interpretative and conceptual development to other disciplines. Thereby it has lost the fruits of some of its most arduous labor and has hardly been given the recognition it deserves.

But we are not particularly concerned with the past; at twenty-one a youth sees only the future. Actually, plant physiology is in a very strategic position. Taxonomy, long primarily a descriptive science, gives indication of adopting experimental methods. Many aspects of ecology are applied physiology. Anatomy is giving increased attention to the function of the structures it describes with such minuteness. Genetics, long a highly developed experimental science, is impinging on physiology. The same is the case with phytopathology and with other branches of botanical science. Plant physiology has an opportunity as never before to play an important part in the development of fundamental concepts resulting from investigations which can be pursued most effectively through cooperative effort. And not only in relation to the botanical sciences can plant physiology be of value, but wherever plants or plant products appear our science has some contribution to make, as in nutrition, chemistry, and medicine.

Plant physiology has thus far been a rather highly specialized science. A specialty is of value only as a part of a larger field. Our situation in plant physiology is very much like that of the early days of railroading. A lot of railroads were built which started nowhere and ended nowhere in particular. It was only after these were built into great systems that we really began to go places. Plant physiology is a highly important link in the larger system of plant biology. We can help that system to grow, and as the larger system grows, we shall grow. As a nation we are inextricably involved in world affairs and in these world affairs food and many other plant products play a vital rôle. This, in part, at least, is the domain of plant physiology. We must contribute more not only to the scientific basis of agriculture and forestry, but also to that vast and rapidly expanding field of chemical industry which depends upon organic carbon. Plants are not only the basis of life; an ever increasing portion of our industry is drawing upon the plant kingdom for its raw materials. And while we are at it, let us not exclude coal and petroleum from our domain. Wherever there is need for carbon compounds the plant physiologist has an opportunity to make a contribution. This applies in the first place to that great array of substances which are used directly as they are derived from the plant such as drugs, oils, lacquers, resins, spices, perfumes, insecticides, beverages, *etc.* The knowledge of most of these materials came to us from primitive man. Science has hardly begun to explore the potentialities of the plant kingdom. Beyond this, however, lie the needs of the chemical industry for all kinds of raw materials in the form of carbon compounds. The much vaunted syntheses of organic chemistry are essentially the juggling

about of organic compounds into forms for which there is a demand. Organic chemists need the raw materials with which to carry out their juggling, and many of these raw materials have some plant as their ultimate source. In a large measure we are the custodians of this branch of knowledge. Within our speciality are the first opportunities to discover the things which other sciences, pure and applied, are seeking.

To the degree that we can make ourselves useful will our science thrive. We are living in the middle of the 20th century, faced with world cooperation and with world competition. In a large measure plant physiology lives still in the last century in respect to its efforts to integrate its results with those of other sciences and industry. In regard to demonstration of the usefulness of our science to society we have been outstripped by many other sciences, such as chemistry. In the case of the latter, the pure or fundamental aspects of this science have certainly not suffered because of contact with practical applications. On the contrary, the fundamental aspects of chemistry have if anything been furthered through these associations. And certainly so far as education is concerned, this has been quickened by virtue of the fact that so large a number of enterprising young people can look forward to a career in chemistry.

A characteristically youthful reply to much of what has just been said is: "How are we to get the support for such undertakings? Chemistry can do these things because of its great financial resources." To this there is but one answer. We shall have to do as the chemists did. We shall have to earn it.

It is not surprising that youth should be inordinately concerned with financial support of its scientific efforts. He is taught the ripened fruits of scientific work, rarely anything about the arduous labors which went into its culture. He sees fully grown industries, rarely their humble beginnings. His attention is constantly drawn to the full grown oak, rarely to the acorn. As a matter of fact, financial support is one of the last things that needs concern. Far more important is whether there is a discovery, a new observation, a novel relation between known facts, or a new product which merits support. Think of the astonishingly simple conditions surrounding the discovery of penicillin, and how rapidly the world was clamoring to give it support.

The basis for the support we have had thus far was in large measure earned by our predecessors who founded our science. We must re-earn our inheritance in order to possess it. But more than that, if our science is to grow we must add to this inheritance so that a greater estate can be passed on to our progeny. In order to accomplish this we must definitely adopt means which are in conformity with a changing world. In the new world order we cannot simply take it for granted that old methods will continue or suffice to meet our new responsibilities.

In the past we have lived primarily upon endowments and various forms of tax support. Youth takes for granted the living conditions under which

it has been raised, and usually regards them as the best imaginable, particularly if they have provided for him comfortably. There is no doubt that these methods of support have proved to possess many advantages. The urge has been to get more and more of the same kind of support. That is perfectly understandable and proper. We have, however, been inclined to overlook or to disregard the monastic and bureaucratic characteristics and influences which are inherent in this type of support. May we not with profit examine other methods of support for scientific research which have been evolved by some of our sister sciences and which have enabled these sciences to make such remarkable demonstrations of their usefulness to society? I refer to the general type of endeavor which has been developed by the Mellon Institute, and the Illinois Institute of Technology, and which in one form or another is being followed by many educational and scientific institutions, both here and abroad. The aim has been to integrate more directly scientific research and industry. The productiveness of these undertakings has been one of the outstanding organizational accomplishments in science. The public and the industry have both profited. Fundamental science has profited. Educational institutions and methods have been quickened and thousands of jobs have been created for ambitious young men and women. Thus far participating institutions have centered their efforts largely around the various branches of engineering, chemistry and physics. Certainly plant physiology has as valuable contributions to make to mankind. They are, no doubt, of a different nature, but they are quite as important.

It must be clear at the outset that in any arrangement involving industrial support high scientific and academic standards must be safeguarded. There is already available sufficient administrative experience to guide us through most of the shoals of such a course. All new ventures raise new problems. To scientists new problems are not a cause for despair, but rather a challenge.

Plant physiology deals with things most closely associated with the basic needs of man. Many of us could, I am sure, get along without a radio, without many electrical gadgets, and without cosmetics. But we could not get along without food, without houses and without the inspiration of a garden or of green fields and forests. Man is fundamentally a product of Nature. In the innumerable contacts of this man with the highly artificial environment he has created, plant physiology can be of inestimable value in helping him to lead a more healthful and rational life. In this connection there are many agencies and industries in need of plant physiological knowledge and skill. To the degree that we give and make ourselves useful to society will society support us.

Academically it may be argued, of course, that fundamentally it makes little difference how our support is obtained so long as the great domain of plant physiology is extended. It is definitely not my intention that cooperative support should in any sense replace the endowments and tax

support upon which we have relied. It is rather with the idea of broadening the base of our support, of enlarging the sphere of our influence that these suggestions have been made. As biologists, we are aware of the significance of the biocoenose for any particular organism. By the same token we cannot disregard our place in the mutual relations of a complex and changing society. The strength of a democratic society lies in the diversity of its social and intellectual institutions and in its ability to adjust itself to changing conditions.

The coming of age of any human or of any institution marks an important epoch. It means that the hazards of youth have been successfully overcome, and that there is sufficient virility and strength to look with confidence to winning out in the future struggle for existence. Our society has demonstrated its virility and its strength. It has lived through a desperate depression and a devastating war. It has the strength of youth to strike out in new directions and some background of experience to guide it. It has a sound inheritance, drawn from all parts of the world and from many branches of learning. Our society enters manhood over the threshold of a new era in the history of the world, equipped to carry its full share of responsibility, endowed with enterprise and strength, and, I am sure, with the whole-hearted support of its entire membership.

CARNEGIE INSTITUTION OF WASHINGTON
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PHOTOCHEMICAL REDUCTION OF CHLOROPLAST GRANA

S. Aronoff

(WITH SEVEN FIGURES)

Introduction

It has long been known that disintegration of green leaf tissue results in almost complete disappearance of photosynthesis. Indeed, any drastic treatment, of a type amenable to the isolation of various enzymatic systems, as intensive dehydration (above or below freezing), low temperature buffered extraction *et cetera* are of no avail. Attention has therefore been focused on some of the larger subcellular units known to function in the process, such as the chloroplasts.

Englemann (**6**) showed, by means of bacteria motile only in the presence of oxygen, that oxygen is evolved only by the chloroplasts within cells. Later, Beyerinck (**3**) used bacteria which can luminesce only in the presence of oxygen to demonstrate the evolution of oxygen from isolated chloroplasts. The maximal sensitivity of the latter method corresponds to the determination of oxygen having a partial pressure of 10^{-8} mm. Hg. The sensitivity of the motile bacterial method is not known, except as the vague reference of Pfeffer (**23**), as a method capable of detection of one billionth of a mg. of oxygen. Using a method (quenching of a phosphorescent dye by oxygen) only slightly less sensitive than that of the luminous bacteria, but better adapted for routine quantitative measurements, Franck (**11**) has recently measured quantitatively the output of oxygen from isolated chloroplasts under various conditions.

Nevertheless, the activity of isolated chloroplasts, prepared by disintegration of leaf tissue, and differential centrifugation of the filtered mass, has been so low that until recently manometric methods could not be used to detect gas exchange. For contemporary routine biochemical investigations, therefore, their activity was nil. It was therefore of considerable interest that Hill (**14**) was able to show that on the addition of ferric oxalate to a solution of freshly prepared chloroplasts, considerable oxygen evolution occurred on illumination. Ferric ion was shown to be reduced to ferrous, and water was oxidized to oxygen. Quantitative measurements showed the reaction to follow the equation:

$$4\ Fe^{+3} + 2\ HOH \longleftrightarrow 4\ Fe^{+2} + 4\ H^{+} + O_2.$$

Holt and French (**20**) showed that the reaction could be followed titrimetrically for the H^{+}.

A very much further advance was made when Warburg and Lüttgens (**24**) demonstrated that an analogous reaction occurred using p-benzoquinone, not only with the chloroplasts, but with chloroplast grana. The reaction followed the equation:

$$2\ \text{quinone} + 2\ HOH \rightleftharpoons 2\ \text{hydroquinone} + O_2.$$

The advance was of a two-fold nature: first, the complicated group of reagents required in the Hill reaction was replaced by a single substance; and second, the use of an organic oxidizing agent demonstrated the generality of the reaction. ARONOFF (1) further emphasized the generality of the reaction by the use of various types of quinones; *e.g.*, naphtho- and anthraquinones, to give the reaction with the grana, though at different rates, roughly proportional to their redox potentials in strong light.

BOICHENKO (4, 5) using a solution of 0.1 per cent. fructose in almost saturated basic Mg. acetate, has reported the evolution of oxygen from dried, as well as freshly isolated, chloroplasts. Unfortunately, we have been unable to confirm his results with manometric technique (the author used the leuco-dye method).

The work of FAN *et al.* (8) is also of interest since these workers have reported the reduction of benzaldehyde to toluol and the corresponding evolution of oxygen in light from green algae in the absence of external CO_2.

The work to be described here forms the basis for the previously reported note. We have found that the reaction with quinone was given not only by the grana, but also by a clear solution containing chlorophyll in only minute amounts, and by lyophilized preparations of both. The subsequent sections therefore deal with (I) the method of preparation of the granules, solution, dried material, and experimental methods; (II) the rate of deterioration of the granules; (III) the rate of reaction of the various substances, as well as that of a leaf disc from which they were made; (IV) the action of poisons and narcotics; and (V) relation of the reaction to photosynthesis.

Methods

PREPARATION OF MATERIAL

GRANA.—In the following the use of the word "grana" is restricted to the product obtained under the conditions described, and is not intended to denote any morphological entity in normal tissue. This is a question that is, apparently, still open for further investigation. Market spinach was used as the source of granules. It had been hoped that tobacco chloroplasts, which can be prepared more abundantly (per weight of fresh tissue), and for the leaf of which more analytical data exist, could be plasmoptized and permit a comparison of chloroplast and granular reactions. Unfortunately, these chloroplasts do not disintegrate to any appreciable extent even in 48 hours of standing in distilled water, and spinach was found to be a ready source of "grana."

Spinach was purchased at a local market and was consistently of a better quality than that obtainable in a dozen or so other establishments in the immediate vicinity. It was equilibrated in a closed chamber to the temperature of the cold room (2–3° C.) within an hour, and all subsequent operations carried on therein, up to the moment of manometer manipulation.

Leaf lamina were separated from stems and large veins in amount sufficient to fill the container of a Waring blendor without packing (ca. 100 gm. of turgid tissue); 175 ml. of phosphate buffer (pH = 6.5, 0.05 M) were poured into the blendor (just enough to cover the blades). The blendor was connected with a variable resistance and run 15 minutes at 55–57 volts in order to minimize the foaming. Below this voltage shearing action was insufficient. An additional 15 minutes were permitted for condensation of the foam to a dark green solution which was then filtered through two inches of cotton into centrifuge tubes. Although 15-minute centrifuging at 45 g. is sufficient to remove all chloroplasts, this material was run at 125 g. The resulting solution was relatively dark green, containing particles of a size at the limit of high-power microscopic resolution (ca. 0.1 μ) and having a chlorophyll concentration of ca. 0.1 mg./ml. The solution thus obtained was transferred into the celluloid tubes of a high-speed centrifuge and run at 6700 g. for 15 min. This is sufficient to precipitate almost all the grana and must be excessive, since the grana are rather tightly packed. The supernatant solution, yellow-green in color, was preserved for further work (see below). The grana themselves were washed twice while packed and then disintegrated with a stirring rod using a minimal amount of buffer. One hundred to one hundred forty ml. of initial granular material were thus concentrated to ca. 20 ml., the chlorophyll content of which normally ran ca. 0.75 mg./ml., often 1.0 mg./ml., and could be made as high as 2.5 mg./ml. The material thus obtained was used directly for experiments. The yield of material (in terms of chlorophyll) was 2–3 times as great for winter spinach (Dec., 1945) as spring (May, 1946), possibly because of the greater friability of the winter chloroplasts (whose yield, therefore, was negligible).

Solution.—The yellow-green solution obtained from the high-speed centrifugation was clear, but showed a pronounced Tyndall effect. This material is referred to as "solution." It was dialyzed against distilled water for 36 hours, whereby additional chlorophyll-containing material was precipitated, and the yellow-green solution though very clear showed a very slight Tyndall effect. This material is referred to as "dialyzed solution." A hand spectroscope still showed the primary red absorption band of chlorophyll on looking through a 10-cm. path. The yellow color is due to the presence in high concentration of a yellow protein with maxima at 337 and 270 mμ (with a relative log K of 0.360 and 0.415 respectively). It is precipitable with ammonium sulphate and redissolves in saline solution. The yellow prosthetic group is so tightly bound to the protein, that no color is lost on dialysis. Although spectral indications would tend toward a flavone pigment, and a green coloration with ferric chloride shows the presence of hydroxyl groups, the characteristic orange color produced on reduction of a flavone or flavonone does not occur here. The high concentration is inferred from (a) the yellow color of a material absorbing in the far ultra-violet, and (b) the fifty-fold dilution required to obtain spectral measurement.

Dried.—Both materials described have been dried in a lyophilizer made

by connecting in series a pump, trap in CO_2 solvent bath (in this case ethyl alcohol), and an Erlenmeyer having a male ground joint. The desiccation of 50 ml. of material, frozen before desiccation and kept frozen by the rapid evaporation of water, was effected overnight.

Reagents

1,4-benzoquinone was obtained from the Eastman Kodak Co. The material as obtained is sufficiently impure so that poor or negative results are obtained. A single sublimation is sufficient purification.

Potassium 1,4-naphthoquinone 2-sulphonate was prepared from α-naphthoquinone according to the method of Fieser (**9**). It was twice recrystallized.

Potassium 1,2-naphthoquinone was prepared in similar fashion according to Fieser's method, and twice recrystallized according to his directions.

Potassium 9,10-anthraquinone 4-sulphonate was obtained through the courtesy of Dr. W. Nudenberg of this department. It was found that the corresponding sodium salt, obtainable through the Eastman Kodak Co. is satisfactory as received.

Other compounds as poisons, narcotics, and additional substrates were used without recrystallization. Chlorophyll a + b was prepared according to the method of Mackinney (**22**) except for the drying of the product, which was performed in hi-vacuum, rather than by desiccator. It is believed that this is of value, since this material, after four years in a cork-stoppered tube at room temperature, still shows a good phase test.

Experimental method

Gas exchange was measured manometrically in the usual fashion, using either Warburg or differential manometers, as the occasion required. Purified nitrogen (Ohio Chemical) was passed through alkaline anthraquinone-sulphonate to remove oxygen (ca. 0.1%), although this precaution was found to be unnecessary when using benzoquinone, and provided the standard atmosphere for the reaction. In filling the differential manometer it was found convenient to employ a three-way stopcock attached to the two manometer arms, so that equal pressure could be maintained in both arms. Following the 15-minute gas introduction, the inlet tube *above* the stopcock was removed, the vents on the manometer vessels closed, and the entire system permitted to attain equilibrium for 5 minutes. The manometer stopcocks were then closed and the three-way stopcock removed.

A 2000-watt tungsten lamp served as light source. The beam was spread by means of a cylindrical lens, collected, and reflected up through about four inches of water into the vessels. The light intensity in the center of the system at the level of the vessel bases was 19,000 lux. Inasmuch as it diminished somewhat on both sides, corrections were made in all runs on the basis of a linear relation between the light intensity and the rate of reaction (see section III). Diminution of intensity, when required, was accomplished by means of calibrated metal screens.

Total chlorophyll was measured by means of a Klett colorimeter, using Corning filter #2403. With an abscissa of γ of chlorophyll/ml. (chph./ml.) and an ordinate of density, the equation of the straight line was $y = 8.52\,x$. This equation is valid for $x \leq 15$. In actual practice, the suspension of grana was diluted one to ten with acetone, the mixture again diluted one to ten with acetone and then centrifuged 5 minutes at 100 g. to give a clear solution.

Rate of deterioration of granules

The data of both HILL and SCARISBRICK (**17, 18**) and KUMM and FRENCH (**21**) indicate a substantial rate of loss of activity of chloroplasts although the temperature of storage is not indicated in either case. The half-lives are of the order of 1.5 and 3 hours. Granules, stored at 2–3° C., were (assuming cold storage of chloroplasts) considerably more stable (see fig. 1),

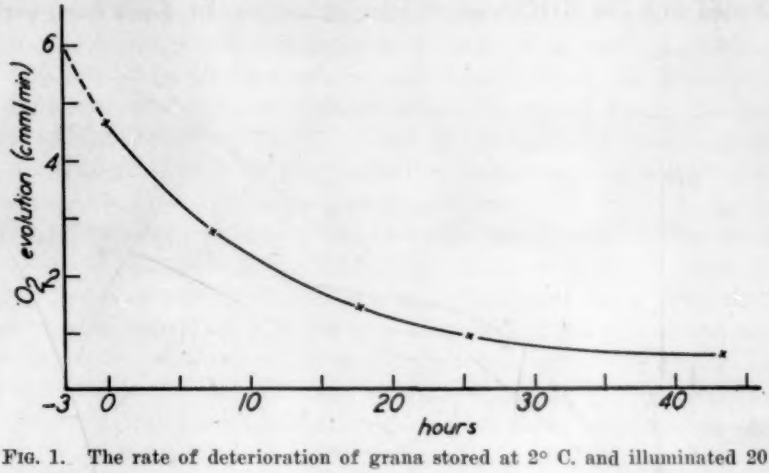


FIG. 1. The rate of deterioration of grana stored at 2° C. and illuminated 20 minutes at 19,000 lux.

the half-life (from the initial measurement) being eleven hours. As with chloroplasts, no extetrnal change in the appearance of the material was noted, though it could possibly have been noted in a change of the fluorescence yield. Because of the shape of the curve, the initial decrease is profound, there being an estimated 25–30 per cent. loss in activity of the isolated material during the time of preparation.

The loss of activity during an experiment was much greater and was presumably due to photolytic effects. Although experiments could be continued for 2–3 hours, the rate fell off markedly after 25–30 minutes.

It was noted, empirically, that the rate of deterioration was also a function of the quality of the material employed, as well as of the season. The figure shown is, therefore, of average material.

Rates of reactions of substances

The reaction had previously been shown to cease in boiled material, to decrease with time as material "deteriorated," and to diminish on narcotiza-

tion. Since each of the processes removes chlorophyll from the reaction, the first two by precipitation as colloidal, insoluble material, the latter by surface coating, it was desirable to show that the reaction did not occur with pure chlorophylls a + b in true solution. The reaction was carried out in both 95 per cent. ethyl alcohol and benzene saturated with water, with and without the addition of amino acids in both cases. No evolution of oxygen was noted. It is, of course, possible that aside from the twenty-fold reduction in amount of water present, and the replacement of hydration by alcoholation, that some essential component has been omitted.

Leaf disc

The amount of photosynthesis of a leaf disc cut from material investigated was determined at various light intensities. For this purpose the leaf was floated in 0.1 M $KHCO_3$ in an inlet atmosphere of 4 per cent. carbon

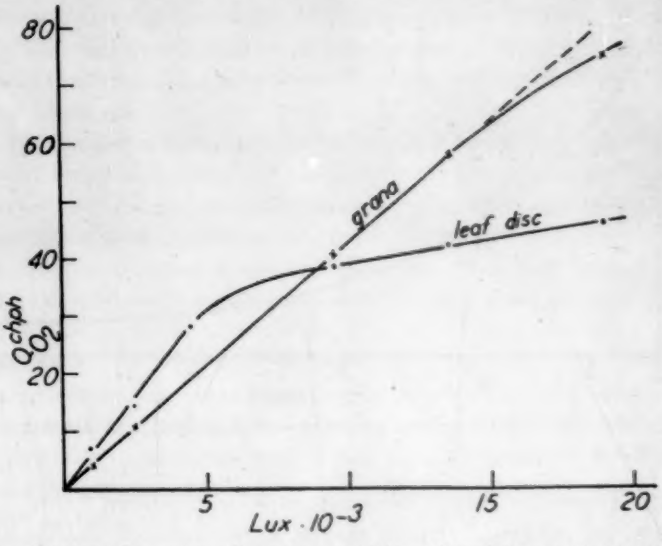


Fig. 2. Comparative rates of oxygen evolution of leaf disc with bicarbonate and grana with quinone.

dioxide, 96 per cent. nitrogen, and ca. 0.1 per cent. oxygen. The results are shown in figure 2. Among points to be noted are (a) the early light saturation, normally expected at ca. 17,000 lux for this material, and indicating some physiological disarrangement (of the time involved in the cutting, transit, marketing, and measurement, only the last is known); there is also a possible deficit of oxygen; (b) the low rate of evolution of oxygen; (c) the higher efficiency of the leaf at low light intensities than that of the grana (for the same amount of chlorophyll), especially in view of the very low redox potential of the oxidant in the leaf (carbon dioxide) and the high potential in the grana (p-benzoquinone). The quantum yields in our material would therefore be quite low. The fraction of the light

absorbed by the leaf per unit area is roughly equal to that of the grana suspension in the vessel.

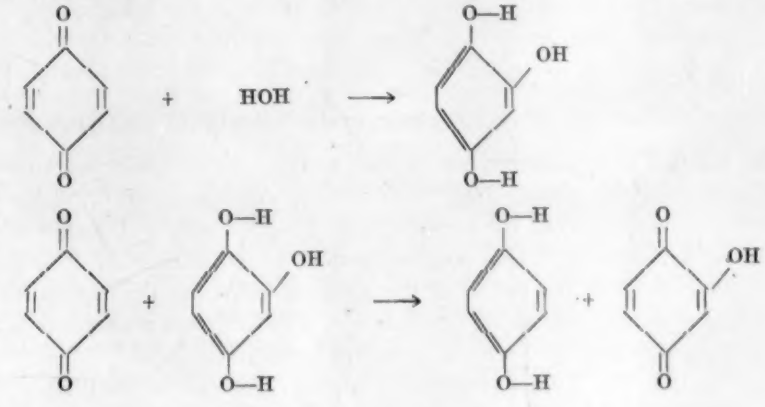
GRANA

Measurements were performed, except in the cases noted, with p-benzoquinone. An excess, ca. 10 mg./5 ml. suspension was used. Using known quantities of quinone, the writer was not able to exceed 35 per cent. yield in a number of experiments, although WARBURG and LÜTTGEN (**24**) report 85 per cent. yields. The cause of the difference is not apparent. It is, for example, necessary that the quinone be purified. Alkalinity, light, *etc.*, cause a solution of purified (sublimed) quinone in Pyrex-redistilled water to turn orange-brown, with a color similar to that of quinhydrone. The deterioration of 1,4-naphthoquinone under similar conditions results in the formation of equal amounts of hydroxy 1,4-naphthoquinone + 1,4-naphthohydroquinone (**9**) which then combine in the usual fashion similar to quinhydrone formation, giving a colored substance.[1] Were this reaction to go to completion prior to the reaction with the chloroplast grana, it would, of course automatically remove 50 per cent. of the initial quinone. If it does not, the final yield with quinone nevertheless represents a competition between the two types of reactions for the quinone.

The rate of the reaction of the grana (fig. 2) with quinone is obviously a function of light intensity. Within the region of 13,000 lux it is linear for this size of granule. There is some indication it varies with the size. Diminution of activity as a function of time at higher light intensities may be correlated to some extent (as with normal photosynthetic systems) with photo-oxidation, but thermal inactivation resulting from the dissipation of the absorbed light energy and internal oxidation-reductions are undoubtedly more prominent factors since the partial pressure of oxygen is rather low.

Rates are plotted as cm. of oxygen evolved per mg. of chlorophyll per

[1] The reaction in the case of 1,4-benzoquinone would then be following FIESER (9):



hour = $Q_{O_2}^{chph}$, and varied between 45 and 100. Inasmuch as WARBURG and LÜTTGENS (**24, 25**) did not state their light intensities, beyond the statement that the reaction was completed within 30–60 minutes, it is impossible to compare results. The low quotient of the leaf disc itself may indicate that the starting material was in part to blame for the low rates. HOLT and FRENCH (**20**) have obtained values of 300–600 with their material using lower concentrations and higher light intensities. Obviously, had we used higher light intensities, we would have obtained higher yields (at least in the case of benzoquinone, though possibly not in the other quinones). Unfortunately, in the most recently available work from this group (**11**) it is impossible to compare any data, since the manner of denoting "activity" was not stated. A more rational "quotient" would thus also require the inclusion of the light factor.

It was of interest to determine the rate where chlorophyll concentrations were identical but particle size different. Chloroplasts were removed by centrifugation at 45 g. for 10 minutes. This has been found empirically sufficient to precipitate algae and pure tobacco chloroplasts quantitatively. By centrifuging an additional fifteen minutes at 45 g., a crop of "large" granules was prepared. The "smaller" granules were precipitated by centrifuging at 125 g. The colorimeter readings on the unextracted suspensions were 430 for the larger and 362 for the smaller, a difference due to the scattering factor. Neglecting the scattering factor (assuming equal light absorption) the rates of oxygen evolution were identical for solutions of equal chlorophyll content. Actually, however, because a larger fraction of the incident light was scattered by the smaller particles, the amount of absorbed light was less, and on the basis of the light absorbed (a quantal basis) their yield would be higher.

In contrast to the experience of HOLT and FRENCH (**20**) who used ferric oxalate, but in agreement with WARBURG and LÜTTGENS (**24, 25**) who used quinone, no carbon dioxide was obtained in the reaction.

Dried granules, resuspended in redistilled water, were found to be slightly more (58 per cent.) than one half as active as the undried material (using identical initial matter). Three days later the same material, kept at 2° C., was inactive. Dehydration is therefore insufficient for preservation.

SOLUTION

The solution is remarkable for its high activity, being about ten times as great as that of the grana; ($Q_{O_2}^{chph}$ = 6–700). Almost constant yields (at 19,000 lux) were observed for periods as long as two hours. Because of the low chlorophyll content it was possible to note a certain amount of destruction of chlorophyll in the course of the reaction, although the general character of the spectrum remained unchanged. The solutions responded to the naphthoquinones, but were erratic in regard to anthraquinone.

The solution, dried in a lyophylizer and redissolved in distilled water, retains its activity for weeks if kept cold while dried. This contrasts with

the marked diminution on storage of dried granules. Using dried solution, in one experiment with benzoquinone a constant rate of oxygen evolution was maintained after the fourth hour (fifteen minute illuminations each hour—total of one hour).

Rates of reaction of substrates

Relation of redox potentials

It was reported in a previous note (**1**) that the rate of oxygen evolution was a function of the redox potential, using a series of homologous quinones. The figure therein is reproduced below (fig. 3). Because of the almost

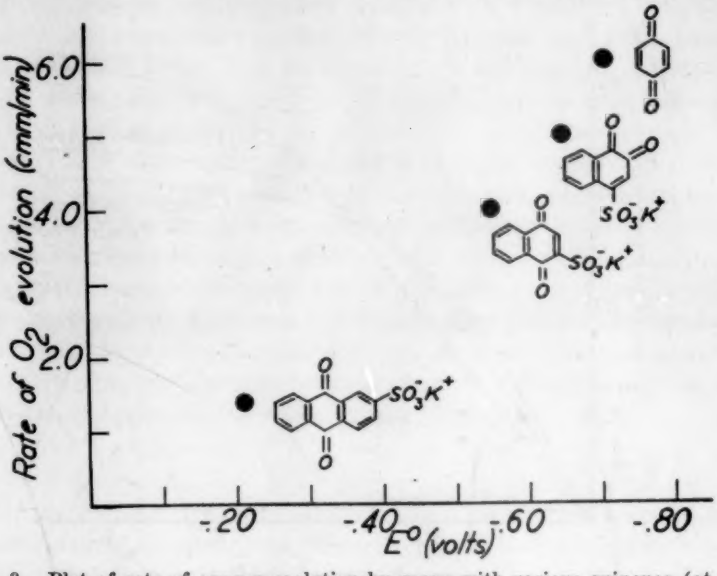


Fig. 3. Plot of rate of oxygen evolution by grana with various quinones (at 19,000 lux) against redox potentials of the quinones (E^{o}.)

linear relationship found between the oxygen evolution and the light intensity in the case of benzoquinone, it was presumed that this relationship held for the other quinones. Furthermore, the results were interpreted in the simplest manner as due to the difference in the rate of the reverse reaction (back reaction) for the different quinones. This explanation was in part based upon the known linear relationship between the redox potentials of leuco dyes and the rate of oxidation (**2**).

Since that time measurements have been carried out with the other quinones, determining the rate of oxygen production as a function of light intensity, the results being shown in figure 4. Measurements were performed as a differential effect; that is, using the same grana preparation, adding a known amount of benzoquinone to one vessel and an equimolar amount of naphthoquinone to the other. Furthermore, the two naphthoquinones were checked against each other in a similar manner; and, of

course, a curve was obtained for benzoquinone alone in the usual manner. Vessels with identical constants were used, and because of the small differences in evolution of O_2, a cathetometer was employed. The results indicate (a) that the order of the rates at low light intensities is the reverse of that at the high (with the possible exception of anthraquinone, where the very early inflection of the curve did not permit the determination of reliable data) and (b) that the curves for the naphthoquinones were linear at only the lower light intensities.

The change in character of the slopes from a straight line to that of a curve appears to indicate that at least one dark reaction occurs in addition to that of the light reaction.

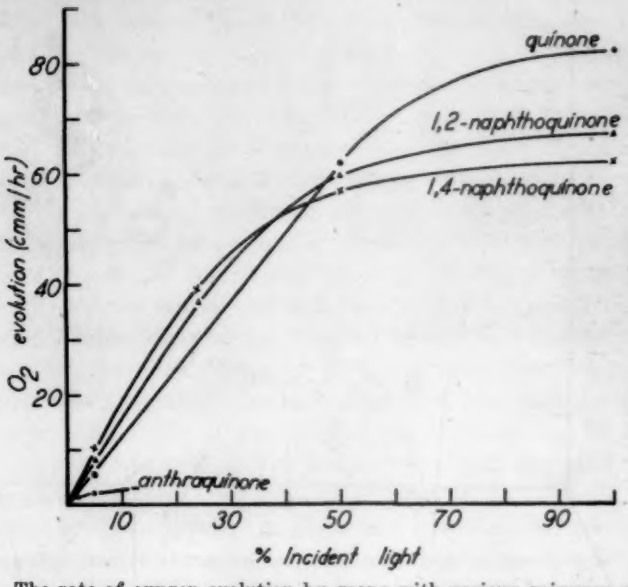


FIG. 4. The rate of oxygen evolution by grana with various quinones as a function of light intensity.

No explanation will be attempted at the present concerning the order of the rates at low light intensity, since this involves further experimentation (as a function of time, *etc.*)

At the higher light intensities there is a choice of two possible explanations. The first, which would be valid if true saturation occurred, would be that of the limitation of the rate by an enzyme with a finite turnover number, worked with insufficient rapidity to keep up with the light reaction. This enzyme would in this instance have different turnover rates for the different substrates and the relation to the potentials indicated would, in a sense, be fortuitous, although in the long run possibly ascribable to the same structural properties which determine potential difference in compounds. The second possible explanation for a diminution in the rate is the increased rate of the reversal of the light reaction with increasing concentration of the

products of the forward reaction. In this instance, saturation, though approached, would never be reached, on the basis of a bimolecular reaction. Unfortunately, the instability of the material at high light intensities does not lend itself to a ready choice between the mechanisms.

Non-quinones

Various other substances have been tried as oxidants; *e.g.*, using salicylaldehyde, a small amount of oxygen is evolved initially, ceasing within a short time, and bleaching of chlorophyll is considerable. Benzaldehyde, although much more susceptible to oxidation, evolves a greater amount of oxygen prior to cessation, than does salicylaldehyde and the chlorophyll bleaching, though considerable, is less severe. Salicylic acid was most effective as a bleaching agent, and no evolution of oxygen was detected. A number of other substances gave very little response: fructose, dehydroascorbic acid, methyl alcohol.

Attempts to obtain a wider indication of the possible effect of potential using a series of dyes (2,6-dichlorophenol indophenol) were abandoned because of the large fraction of light absorbed by the amount of dye required to produce a nominal manometric effect.

The grana decompose urea peroxide in the dark, as does catalase. This probably denotes a high dissociation of the substrate into urea and hydrogen peroxide. Butadiene monoxide (3,4-epoxide, 1-butene) also gave no oxygen.

It is interesting to note that benzoyl peroxide evolved oxygen to some extent, with no observable liberation of gas in the dark.

Algae

Fan *et al.* (**8**) have reported that green algae are able to evolve O_2 in the light and explained their results as the reduction of benzaldehyde to the corresponding alcohol. Their experiments were complicated by (a) the relatively large amount of metabolic carbon dioxide present in the dense suspensions, permitting a release of oxygen due to normal photosynthesis as great as that due to the reduction of benzaldehyde; (b) the apparently usual presence of air during the experiment, resulting in the oxidation of the aldehyde; and (c) the slow rate of entry of the aldehyde into the algal cells.

We have attempted to repeat our experiments on the alga, Scenedesmus. As with the grana, the algae were in an atmosphere of nitrogen (+0.1% O_2), and bathed in a phosphate buffer identical with that used for grana. The sole carbon dioxide available was that produced by anaerobic metabolism. The density of the suspension was far from that resulting in total light absorption. Since an excess of quinone above that of a saturating solution did not increase the rate of oxygen evolution, it was presumed that the rate of quinone entry into the cells limited the oxygen evolution. Under these conditions, oxygen was evolved only in the light. The same algae, under the same conditions, without quinone did not evolve oxygen.

Poisons and narcotics

One of the anomalies of the Hill reaction is the inaction of the usual poisons. Thus, cyanide is of no effect, nor is azide. The former is believed to affect the carboxylating enzyme in normal photosynthesis, and since there is evidence (**14**) to indicate that this enzyme may be located outside the chloroplast, the inaction of cyanide may thus be understood. The same cannot be said of azide[2] which would presumably act on the oxygen-liberating enzyme. Even more pronounced is the lack of activity of hydroxylamine.[1] We had, indeed, hoped for some activity, since, from the point of view of potentials, hydroxylamine should be a good oxidizing agent. Actually, it is a very poor one and is, in fact, used as a reducing agent in normal practice. We have found no evolution of oxygen with this substance, except for a slight initial activity which soon ceased. This activity produced may be due to reaction of the amine with naturally occurring ketones or aldehydes. Thus, the primary action of hydroxylamine in the presence of quinone is to form the dioxime. This is accompanied, however, by considerable evolution of gas, apparently nitrogen (and all, of course, without relation to light or enzymes).

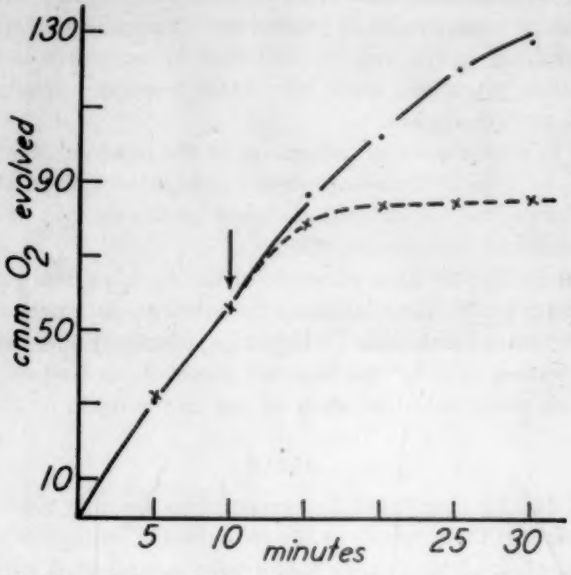


FIG. 5. Inhibition of the evolution of oxygen by grana and quinone by o-phenanthroline. ———— control; - - - - - - o-phenanthroline.

The action of o-phenanthroline was found, in agreement with WARBURG and LÜTTGENS, to be an effective narcotic (fig. 5). The almost complete sup-

[2] FRENCH, *et al.* (12) have noted the action of hydroxylamine and azide as that of poisons. This is at variance with the results of both HILL and SCARISBRICK (16) on chloroplasts, and with ours on granules. They also mention the poisonous effect of fluoride, which HILL and SCARISBRICK found was not a poison. Furthermore they class Duponol (presumably sodium lauryl sulphate) as a poison. Undoubtedly this detergent acts as a surface agent and should therefore be classed as a narcotic.

pression of photoreduction makes it a very powerful inhibitor (although this may be due to its relatively large concentration compared to other narcotics). The substance is termed a narcotic despite its ability to be a poison by virtue of its chelation with ferrous ion, since WARBURG and LÜTTGENS (**24, 25**) found that its inhibiting action can be removed merely by washing and resuspending the grana.

The most common photosynthetic narcotic, phenylurethane, was found, in agreement with HILL's data on chloroplasts to be effective with grana (fig. 6). One notes, however, that with chloroplasts, by using sufficiently high concentrations, almost complete suppression of activity can be obtained with both ethyl- and phenylurethane. In the case of grana, using a saturated solution of phenylurethane, only two-thirds inhibition results. An

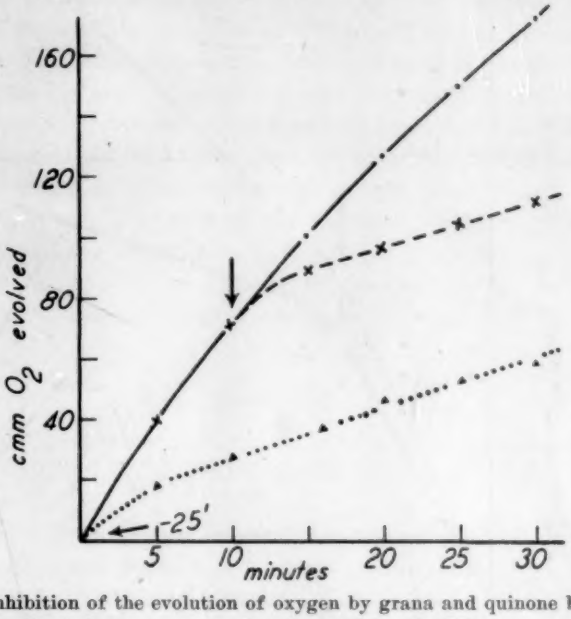


FIG. 6. Inhibition of the evolution of oxygen by grana and quinone by phenylurethane. ———— control; - - - - - phenylurethane (2.0×10^{-2} M); . . . . . phenylurethane (excess; crystals).

additional difference, one which occurs with grana and apparently not with chloroplasts, is the constancy of the inhibited rate. The time effect with plastids is considerable, indicating a considerable diffusion factor through the membrane to the effective loci. The lack of an increased effect of excess phenylurethane (including a previous twenty-five minute incubation) indicates that the two-thirds rate diminution is the limit with this substance.

The principle of narcotic action is well illustrated by comparison of thymol with phenylurethane. Using identical concentrations (1.2×10^{-2} M) in which phenylurethane diminished the control rate by 55 per cent., thymol diminished the rate by 80 per cent. (fig. 7). The distribution coefficient between olive oil and water for phenylurethane is 150, and for thymol, 600

(19, p. 357). On the assumption that the effectiveness of a sub-limiting concentration of inhibitor is then determined by its solubility in the (oily) matrix of the grana, one would have inferred that thymol should be about four times as effective as phenylurethane.

It is to be noted, though possibly at present it is considered coincidental, that the limit of effective narcotic action is attained when the ratio of phenylurethane is one. This implies that with less concentrated grana solutions, less concentrated phenylurethane will be required to produce a given effect. This experiment has not been performed.

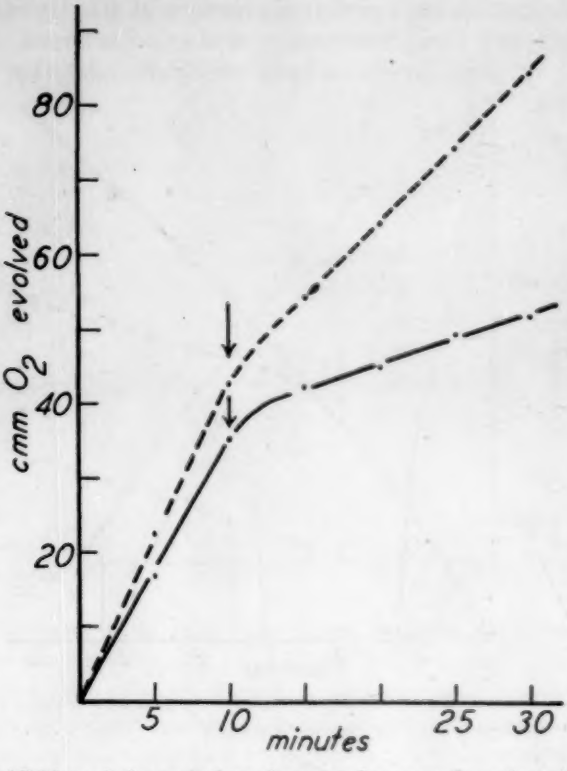


FIG. 7. Inhibition of the evolution of oxygen by grana by submaximal amounts of phenylurethane and by thymol; - - - - - phenylurethane (1.2×10^{-2} M); ——— thymol (1.2×10^{-2} M).

Relation of the reaction to photosynthesis

HILL (14) correlated the activity of iron in photoreduction in his isolated chloroplasts with a specific material reacting as a catalase-like enzyme. That various types of quinones (1, 24, 25), aldehydes, *etc.*, react in a similar manner with products of the chloroplasts, the grana, indicates a generality of the reaction greater than previously thought. It is, of course, possible to write a general equation describing all of the above reactions in a manner

identical to the generalized equation for photosynthesis suggested by van Niel, *viz.*

$$2B + 2HOH \xrightarrow[chph]{light} 2BH_2 + O_2$$

In particular the oxidant B is represented by relatively strong oxidizing agents, as ferric ion, or quinone, whereas in normal photosynthesis, the oxidizing agent is the very weak carbon dioxide. In both cases the oxidant is water, although this has not been shown for photoreductions where a reducing agent, in addition to that of water, is required.

It is not possible to say at present, however, that this reaction is the "last half of photosynthesis." Certainly it is not the "first half" since this occurs in large measure outside the chloroplast. The lack of effect of the poisons (with the possible exception of o-phenanthroline) indicates that the normal enzymatic system responsible for the release of oxygen is not present, or at least is inoperative under the conditions used in the present work. Indeed, as indicated previously, we cannot even be certain that the enzyme(s) involved in the stabilization of the primary photolytic products is operative. The only mechanism of which we may be certain is the fission of water in the light reaction; and it is this step, the "middle" of photosynthesis, which the reactions may have in common.

The nature of the transfer of light to chemical energy is, however, of the utmost importance and significance, and therefore worth continued study.

Summary

1. Methods of preparation of grana and of a clear solution which can be used for the evolution of oxygen by photochemical reduction of quinones have been described.

2. The rate of deterioration of the grana, stored at 2° C. is such that a half-life of about eleven hours is indicated. Approximately 25–30 per cent. of the activity is lost in time of preparation.

3. The rate of oxygen evolution with grana and using quinone is only a tenth that of the solution (at maximal light intensity) based on the amount of chlorophyll. The solution is much more stable in light than grana. Both lyophilized grana and solution are active on rehydration. The lyophilized solution is more stable than the grana.

4. The rate of oxygen evolution varies with different quinones (benzoquinone, 1,2-naphthoquinone, 4-sulphonate, 1,4-naphthoquinone 2-sulphonate, β-anthraquinone sulphonate). At lower light intensities the order of rates is the reverse (with the possible exception of anthraquinone) of that at the high light intensities. Saturation, or an approach to it is indicated at high light intensities. Various other substances give small effects: benzaldehyde, benzoyl peroxide, and salicylaldehyde. Others give very little effect: fructose, and dehydroascorbic acid. Others which might have been

expected to be active give none: butadiene monoxide and salicylic acid. Urea peroxide is decomposed in the dark by both the grana and a solution of pure catalase, indicating dissociation of the urea peroxide to urea and hydrogen peroxide.

5. The photochemical reduction of quinone is inhibited strongly by o-phenanthroline, by phenylurethane, and by thymol. Complete inhibition by phenylurethane does not occur with saturated solution; the degree of inhibition is not increased by incubation or with excess material. Thymol is more fat-soluble than phenylurethane and is more effective as an inhibitor at the same molar concentration.

6. A brief discussion of the relation of photochemical reduction with various substances by grana to photosynthesis is given.

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AN APPARATUS AND METHOD FOR THE MEASUREMENT OF THE RESPIRATION OF COTTONSEED

A. M. Altschul, M. L. Karon, and P. J. Fynn

(WITH TWO FIGURES)

Introduction

In the course of an investigation to determine the relationship of the respiration of cottonseed to the other processes which take place during storage, studies were made of the effect of moisture content, maturity, and inhibitors on respiration, rate of free fatty acid formation, and pigment changes in the oil. For these studies, a series of measurements was required on about fifty different samples of cottonseed over a period of one year.

In view of the large number of analyses needed, an apparatus designed to obtain accuracy and speed was built to follow the changes of the oxygen and carbon dioxide concentrations in the atmosphere surrounding the seeds. This apparatus and the methods used to make and record the measurements are described below.

Methods

A sample of seeds for which a measurement of the respiration rate is desired is stored in a stoppered flask. The size of the sample and the length of the storage interval are determined by the estimated respiration rate of the seed sample. Since an accumulation of carbon dioxide in the atmosphere surrounding the seeds inhibits respiration (**2**), a period of storage is selected that will not permit the development of more than a 3-per cent. concentration of carbon dioxide in the flask at the time of analysis.

At the conclusion of the storage period, a sample of the atmosphere in the flask is withdrawn and analyzed for both oxygen and carbon dioxide content. This is accomplished by the successive removal of carbon dioxide and oxygen accompanied by measurements of the resulting changes in pressure at a constant volume. The gas sample is first dried, then drawn into a measuring chamber of fixed volume kept at constant temperature, and its pressure measured. The percentage of carbon dioxide is then determined by passing the gas sample several times through a tube of Ascarite,[1] after which it is pumped back into the measuring chamber. The percentage drop in pressure represents the carbon dioxide content of the sample. Oxygen is determined on the same sample by passing the carbon dioxide–free gas several times over a heated copper screen before pumping it back into the measuring chamber. The difference between the percentage of oxygen in a sample of air and in the sample which was analyzed is equal to the percentage of oxygen consumed by the respiring seed. After each oxygen analysis, the copper is regenerated by passing hydrogen gas over the heated oxidized copper screen.

[1] A commercial preparation of asbestos coated with sodium hydroxide.

Apparatus

Figure 1 is a schematic drawing to scale of the apparatus used for the gas analysis, and figure 2 is a photograph of the apparatus. All of the

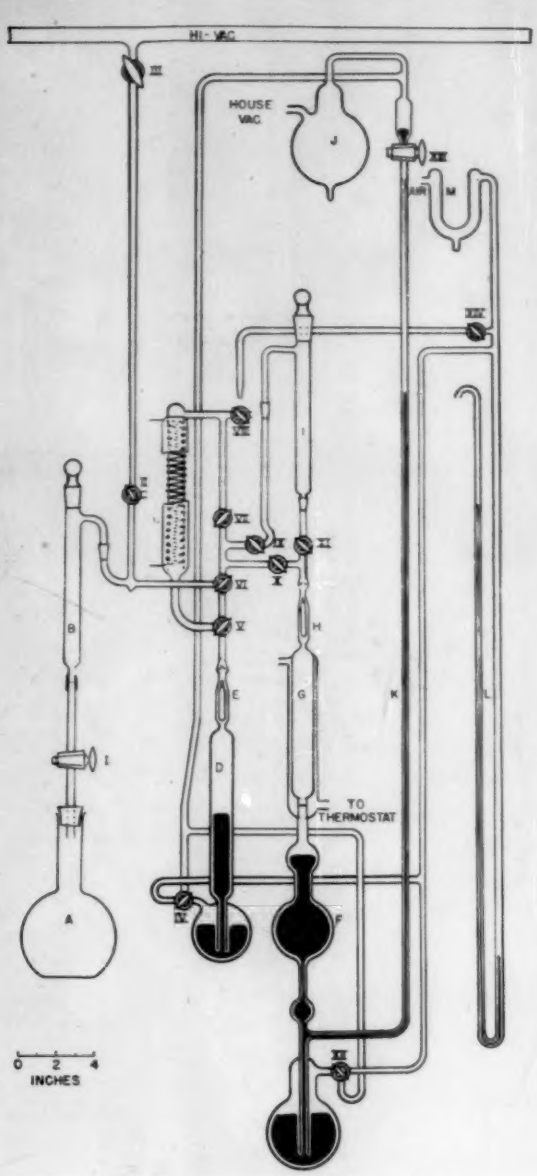

FIG. 1. Diagram of respirometer.

connections in this apparatus were made with standard tapered ground glass joints. Vacuum-tight seals were obtained by cementing the ground glass joints with a phthallic anhydride resin. It will be noted that this

apparatus is similar in many respects to the one described by BAMFORD and BALDWIN (1). It was found desirable, however, to sacrifice some of the

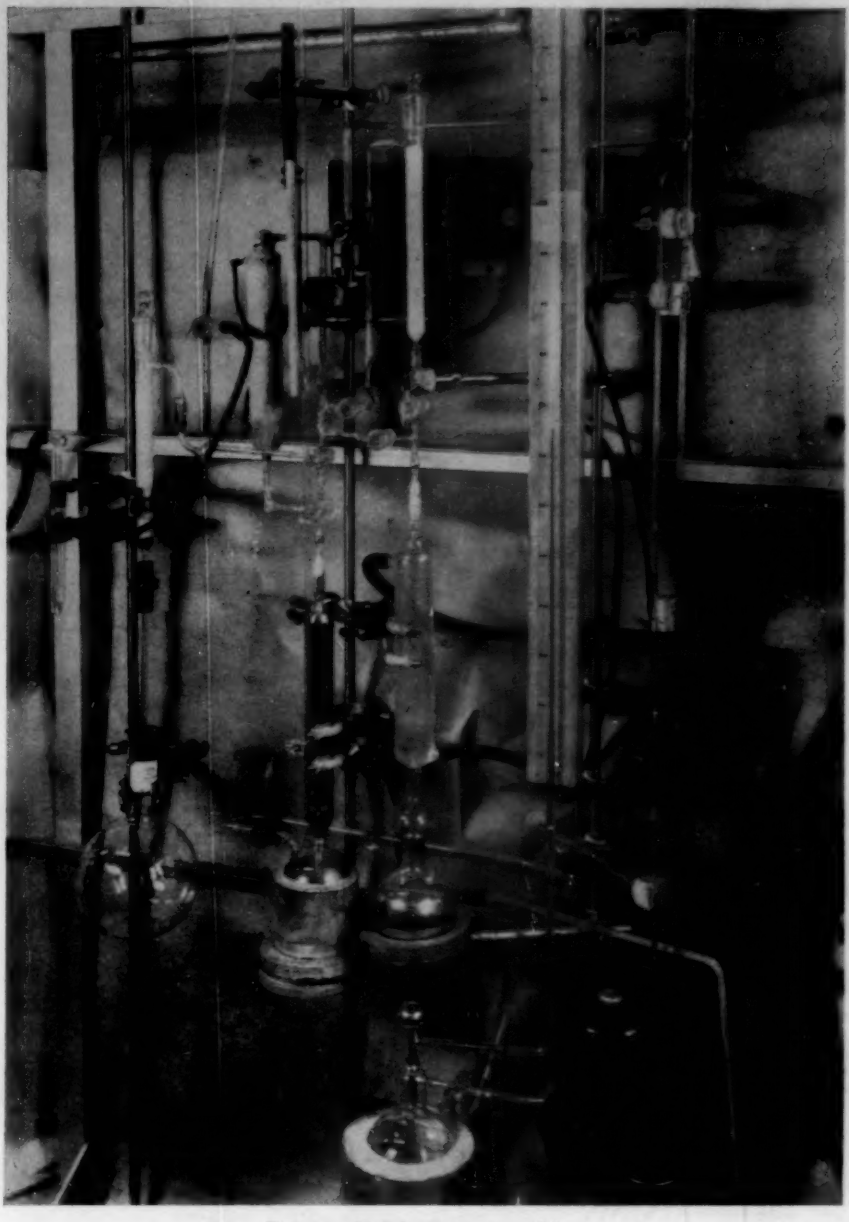

FIG. 2. Respirometer assembly.

accuracy obtained by BAMFORD and BALDWIN in favor of simplification of operations to obtain the speed necessitated by the number of analyses to be

conducted. Nevertheless, almost negligible difference existed between the oxygen content of air found with the apparatus used in this investigation ($20.93 \pm 0.05\%$) and that found by BAMFORD and BALDWIN ($20.94 \pm 0.04\%$).

Method of analysis

Description of the procedure followed with a typical gas specimen will serve to illustrate the method of analysis. The symbols used refer to those indicated in figure 1.

MEASUREMENT OF THE INITIAL PRESSURE

The respiration flask, A, is connected by paraffin-covered rubber tubing to the tube B which contains phosphoric anhydride layered on glass wool. This tube serves to remove water and volatile bases. With stopcock I closed, the entire apparatus is evacuated to approximately 0.05 mm. pressure by means of a "Megavac" pump. The gas sample is admitted slowly into the measuring chamber, FG, through B and the system of stopcocks I, VI, X. Stopcocks X and XI are closed; mercury is forced up through bulb, F, to the mark in the capillary tubing; and the pressure on the manometer, K, is read to the nearest half millimeter. The quantity of gas admitted is such that the initial pressure is slightly less than atmospheric pressure. The chamber, G, is maintained at 26° C. by circulating water from a constant temperature water bath through the surrounding jacket; and the vacuum, in the manometer, K, is renewed daily by forcing the mercury up through the stopcock XIII, before closing it.

MEASUREMENT OF PERCENTAGE OF CARBON DIOXIDE

After the initial pressure has been determined, the sample of gas is passed through the tube containing Ascarite, I, into the Toepler pump, D, through the system of stopcocks IX, VI, and V. Both ends of the tube I contain small amounts of magnesium perchlorate to absorb any moisture produced during the removal of carbon dioxide by Ascarite. Compressed air and vacuum (5-mm. laboratory vacuum line) are used to regulate the level of mercury in the chamber, G, and in the Toepler pump. The air, as obtained from the laboratory source, was under 80 pounds' pressure. This pressure was reduced by the use of the bleeding stopcock XIV, to that required as measured by the manometer, L. The float value, H, prevents mercury from rising into the capillary lines. A similar valve, E, above the Toepler pump affords protection to the capillary lines above the pump.

When all of the gas has been forced out of chamber G into the Toepler pump, the flow is reversed and the gas is pushed back again through the Ascarite absorption tube. The process is repeated twice more, so that, in all, the sample of gas is passed over the Ascarite six times. At the end of the sixth operation, all of the gas will have been forced out of the Toepler pump, and the level of the mercury in the measuring chamber is set at a point below bulb F. The gas remaining in the absorption tube, I, and in

the capillary lines is pumped back into the system FG by the use of the Toepler pump. Four pumpings are sufficient to remove all the residual gas from the lines. With stopcocks X and XI closed, the level of the mercury in the measuring chamber is again brought to the mark in the capillary tube below G and the pressure is measured. The fraction of carbon dioxide in the gas sample is equal to the drop in pressure divided by the initial pressure.

Measurement of percentage of oxygen

Oxygen is removed from the carbon dioxide–free gas by passing the gas over the heated copper screen in tube C into the Toepler pump through the system of stopcocks X, VII, and V. Tube C is electrically heated by means of 30 feet of size 22 Chromel-A wire (0.999 ohm per foot) wound around the outside of the tube. Fifty volts A.C., taken from a variable transformer, are sufficient to give adequate heat for both the removal of oxygen during the analysis and for the subsequent reduction of the copper oxide by hydrogen. The operations used for the removal of oxygen in the sample and for the return of all the residual gas to the measuring chamber are similar to those carried out for the determination of carbon dioxide. When the analysis of the sample has been completed, the copper surface on the screen is renewed by passing hydrogen gas into the heated tube, C, through the system of stopcocks II, VI, V, and VIII.

The drop in pressure resulting from the removal of oxygen in the sample divided by the initial pressure is equal to the fraction of oxygen in the sample. The change in oxygen pressure due to the consumption of oxygen by the respiring seeds is obtained by subtracting the percentage of oxygen obtained by analysis from the oxygen content of the air (20.93%).

Typical results

A set of typical recordings of experimental data and calculations of the results of the gas analysis are presented in table I. In the case of analysis No. 1, the oxygen was first removed, followed by carbon dioxide; in the second analysis the order of removal of the gases was reversed. It will be noted that the order of removal of the gases has no significant effect upon the accuracy of the results.

TABLE I

Measurements and calculations of the results of a typical gas analysis

Analysis number	Pressure			Carbon dioxide evolved	Oxygen content	Oxygen consumed
	Initial	After oxygen removal	After carbon dioxide removal			
	mm. Hg.	*mm. Hg.*	*mm. Hg.*	%	%	%
1	766	610.5	605.5	0.65	20.30	0.63
2	739	584.0	734.5	0.61	20.36	0.57

Starting with data of this type, it is possible to place the results of all respiration experiments on a comparable basis by reducing the analytical values for carbon dioxide and oxygen to the number of cubic centimeters of gas at standard temperature and pressure that is evolved or consumed per day by one gram of seed (moisture-free basis). To effect this transformation, it was necessary to measure the volume of the free air space in the respiration flasks, so chosen that the volume of free space was constant for each size of seed sample. For a 10-gram sample of seed the air space was 1108 cc.; for a 25-gram sample, 1079 cc.; and for a 50-gram sample, 1032 cc. Corrected to standard conditions, the volume of gas in the above flasks was 1022 cc., 995 cc., and 953 cc. for 10-, 25-, and 50-gram samples, respectively.

The complete calculation of the respiration rate of the sample illustrated in table I is shown in table II.

TABLE II

CALCULATION OF RESPIRATION RATE

RESPIRATION INTERVAL	WEIGHT OF SAMPLE	MOISTURE CONTENT	WEIGHT OF DRY SEED	CARBON DIOXIDE EVOLVED	OXYGEN CONSUMED	CARBON DIOXIDE EVOLVED	OXYGEN CONSUMED	CARBON DIOXIDE EVOLVED PER GRAM PER DAY	OXYGEN CONSUMED PER GRAM PER DAY
days	*gm.*	%	*gm.*	%	%	*cc.*	*cc.*	*cc.*	*cc.*
39	50	10.1	45.0	0.63	0.60	6.0	5.7	0.0034	0.0032

Summary

1. An apparatus, developed for the determination of the respiration rate of cottonseed, is described. Carbon dioxide and oxygen are successively removed from the gas sample, and the changes in pressure of a fixed volume of the sample, occasioned by removal of the gases, are used to calculate the percentage of carbon dioxide and oxygen in the sample.

2. The results of the respiration rate measurements are expressed in terms of the number of cubic centimeters of carbon dioxide or oxygen, at standard temperature and pressure, evolved or consumed per gram (moisture-free basis) of seed per day.

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THE RESPIRATORY PROCESSES IN PLANT CELLS IN RELATION TO THE FORMATION OF COACERVATES

J. Dufrenoy and H. S. Reed

(with two figures)

Life depends on the existence of a water phase within the cytoplasm. Generally the water phase, as represented by the vacuolar solution is optically empty: in whatever condition the solutes are, they are hydrated and randomly distributed so that they appear to be in solution. The vacuolar solution, however, may itself become the site of a separation of phases through coacervation, as we intend to show by the data to be presented.

Coacervation may occur in the vacuolar solution of plant cells under the influence of various agencies apt to unbalance cell respiration, such as zinc deficiency, virus or fungal infection. Auto complex coacervates, made up of a central core of polymerized phenolic compounds, surrounded by phosphatides, once formed, appear as permanent structures in the cell and have frequently been misinterpreted by observers. Recent advances in cytochemistry emphasize that the vacuole is the reaction chamber of the cell. The process of coacervation in it affords an unusual opportunity to visualize one of the delicately balanced equilibria so essential for life (**13**).

The polyphenols, chiefly catechol, which are so widely and almost universally distributed in the vacuolar solution of plant cells, have during these recent years been recognized as the most important mediators between the metabolites furnishing the hydrogen and the atmospheric oxygen accepting it in the process of aerobic respiration. In the normal "compensated" respiration these compounds are reduced back to the original condition of polyphenols as fast as they are dehydrogenated to quinones. Therefore they retain their original physical as well as chemical properties.

When the respiration is "non-compensated" the phenolic compounds, having been dehydrogenated to quinones, no more have made available to them the activated hydrogen that can reduce them to their original condition (**9**). Instead of being reversibly reduced, they become subject to further oxidations resulting in concomitant changes of their physical conditions. Some of the linkages which should normally have been C–OH linkages become C–C linkages, meaning linkages between the C of different molecules, building large molecular weight units, or polymers, which assume the consistency of a gum, and the color of a pigment—either the brownish-red phlobaphenes, or the vivid red anthocyanols.

The phenomenon of molecular aggregation, or polymerization, was correlated with changes in chemical affinity by Berthelot (**2**), Guldberg and Waage (**8**) and Pasteur (**11**). Bertrand (**3**) applied his newly acquired knowledge of oxidases to explain the concomitant oxidation and polymerization of Boletol and other phenolic derivatives. Combes (**4**) recognized that

when anthocyanic pigments are formed, oxygen is fixed by the organs which become red; then those organs are the site of enhanced oxidative phenomena. Conversely, when anthocyanic pigments disappear, the organs whence they disappear release oxygen. It is possible to demonstrate an aggregation of oxidizable molecules into more or less soluble gummy masses in cells which are the sites of unbalanced or non-compensated respiration. The phenomenon may be correlated with a process of dehydrogenation not only *in vivo* but *in vitro*.

Relation between coacervates and the non-compensated respiration

Diseased tissues which evidence discoloration, absorb more O_2 than normal, and release less CO_2; oxygen acts as a hydrogen acceptor whereby the phenolic compounds become dehydrogenated into pigments. Oxygen, however, is not fixed by the pigment. As phenolic compounds, such as catechol, normally dispersed in solution in the vacuolar sap, are being dehydrogenated, due to non-compensated respiration, both the rH and the pH of the vacuolar solution would shift; correlatively the behavior of the compounds toward water will be altered. Molecules of polyphenols, originally dispersed at random in the vacuolar solution, become grouped into larger units of polymers and those units may not remain distributed at random, but may become more densely congregated at certain loci.

This unequal distribution of phenolic compounds within the vacuolar sap may be achieved through one of two ways:

1. The vacuolar sap in the cell becomes apportioned to different vacuoles, some of which may contain phenolic compounds, others not. In other cases the initially single vacuole containing the whole of the vacuolar solution in the cell may bud out, at its periphery, a number of drops which will become individualized into many small vacuoles. These drops may contain a solution richer in phenolic compounds than the original solution, in which case the central vacuole may be described as secreting or excreting phenolic compounds, or, on the contrary the exudate may be devoid of phenolic compounds, in which case the original vacuole excretes water, and concentrates the phenolic solution.

2. Instead of being achieved through the fragmentation of the vacuole, the concentration of the phenolic compounds can occur in the vacuole itself which retains its full volume, while the phenolic compounds contract spontaneously and become condensed as a mass floating in the vacuolar liquid, now impoverished in colloids.

Many investigators actually stained coacervates with dyes like neutral red, but failed to realize that they were floating in the vacuolar solution. MAST (10), studying the relations between the kinds of food, growth, and structure, reported that *Amoeba proteus*, which had fed for several days exclusively on Chilomonads, contained an extraordinarily large number of spherical bodies which showed a definitely differentiated structure;

namely, a central mass surrounded by a distinct, though fragile, shell which was covered with a thin layer of oily substance. In solutions containing neutral red the outer layer became crimson in color, but the central portion and the shell were not stained by the dye. Coacervates thus express the ultimate effect of a gradient in the distribution of the vacuolar materials. The phenolic compounds as they lose hydrogen develop ability for C linkages; hydrogen ties up into H_2O or H_2O_2; less and less hydrogen is available to redisperse the polymerized phenolic compounds, or rather the dehydrogenase systems (whereby hydrogen should have been made available) fail to perform, either because the active phosphorus linkages have been broken or because the active SH groups have been oxidized to disulfides.

The loosening of phosphorus linkages concomitant with the dispersion of the dehydrogenase systems, provides for a supply of ionic PO_4 which goes into the vacuolar solution, there to become incorporated into the amphoteric lipid complexes at the boundary of the auto complex coacervates.

The non-compensation of respiration, following dispersion of dehydrogenases, lack of active hydrogen for rehydrogenation of coacervated phenols, immigration of PO_4 ions into the vacuoles onto the boundary of the coacervates, can be experimentally induced by treatment with agents such as salicylaldoxime interfering with the normal activity of dehydrogenases.

Coacervates in plant cells

The effect of disrupting the dehydrogenase system was convincingly shown in the experiment where sugar cane was treated with 100 p.p.m. sodium xanthogenate. Within a few hours living cells were in a condition represented by figure 1. Following treatment with the molybdenum reagent, the nuclear mass gave a diffuse reaction for phosphorus, mitochondria were swollen, phenolic material was coacervated within the network of the phospholipid and was floating in the vacuolar sap. This condition can be interpreted as the result of the disequilibrium between the dehydrogenase system and the oxidase system. The former being more sensitive, most reagents are likely to induce a non-compensated respiration as expressed by coacervation. This will explain the common occurrence of coacervates in pathological tissues as will be shown later.

About 1940, in some of the sugar cane fields of Louisiana were first detected a few stalks affected by an infectious disease, which was named "chlorotic streak." Abbott and Ingram (1) obtained evidence that it is a virus disease and showed that it could be transmitted by a leaf-hopper. A red discoloration of vascular bundles at the nodes was revealed when the affected stalks were split lengthwise. Freehand longitudinal sections of the nodes showed in most perivascular cells a large coacervate, the contents of which gave positive reactions for phenolic compounds, and most specifically gave the positive Scudi reaction for pyridoxin (6). The coating of phospholipids separating the inner solution (rich in pyridoxin) from the depleted outer vacuolar solution could be easily demonstrated by the molybdenic reagent.

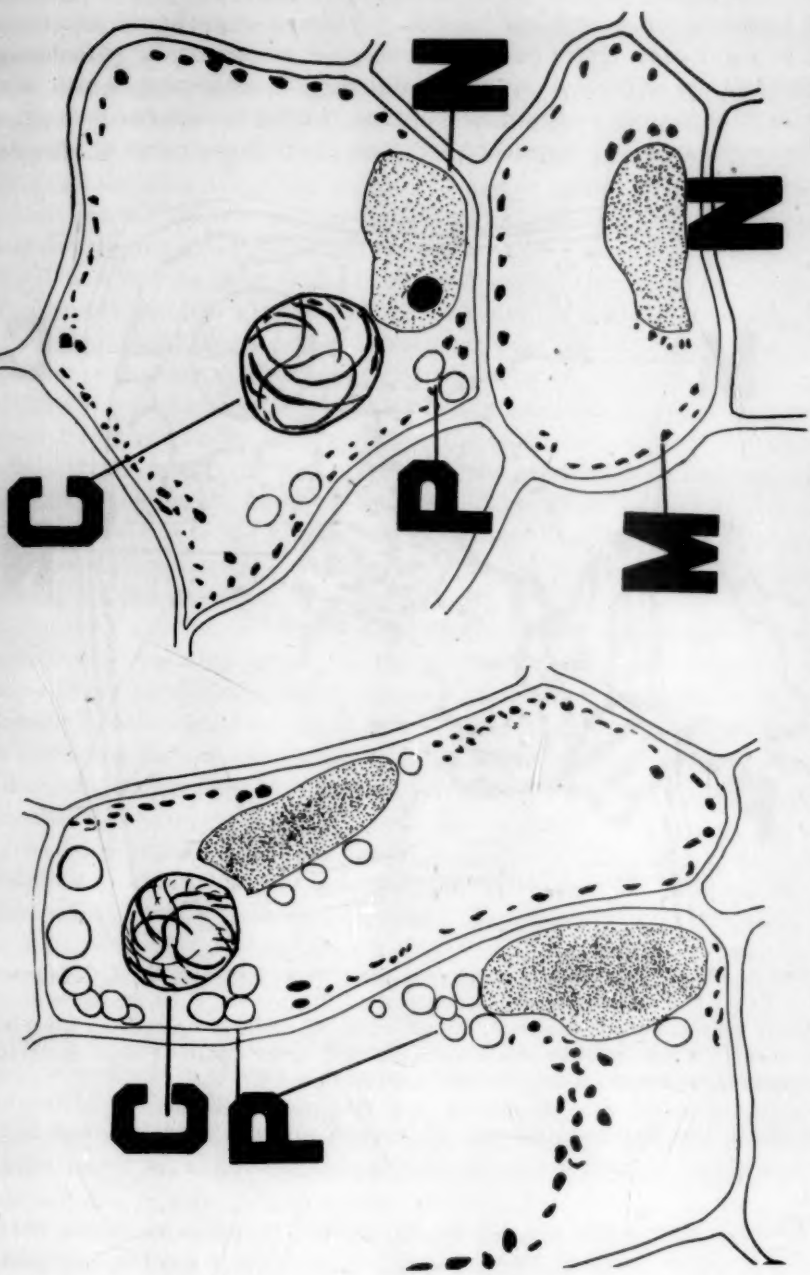

FIG. 1. Cells from a stalk of sugar cane infiltrated with a solution of sodium xanthogenate. These sections were treated with the molybdenum reagent, revealing the nucleoproteins in the nucleus, N, in the plasts, P, and the mitochondria, M, and also the phosphatides at the periphery of the coacervates, C.

These coacervates could also be demonstrated in permanent mounts of sections of tissues fixed in the HELLY's killing fluid, and properly stained with haematoxylin or with acid fuschsin. Ultimate stages of cell disintegration in sugar cane, either due to physiological senescence, or pathological breakdown are expressed by the prevalence of coacervates which may eventually be the only recognizable features. In fact the intermediate phase of the coacervate is so permanent a structure that the inner core of phenolic

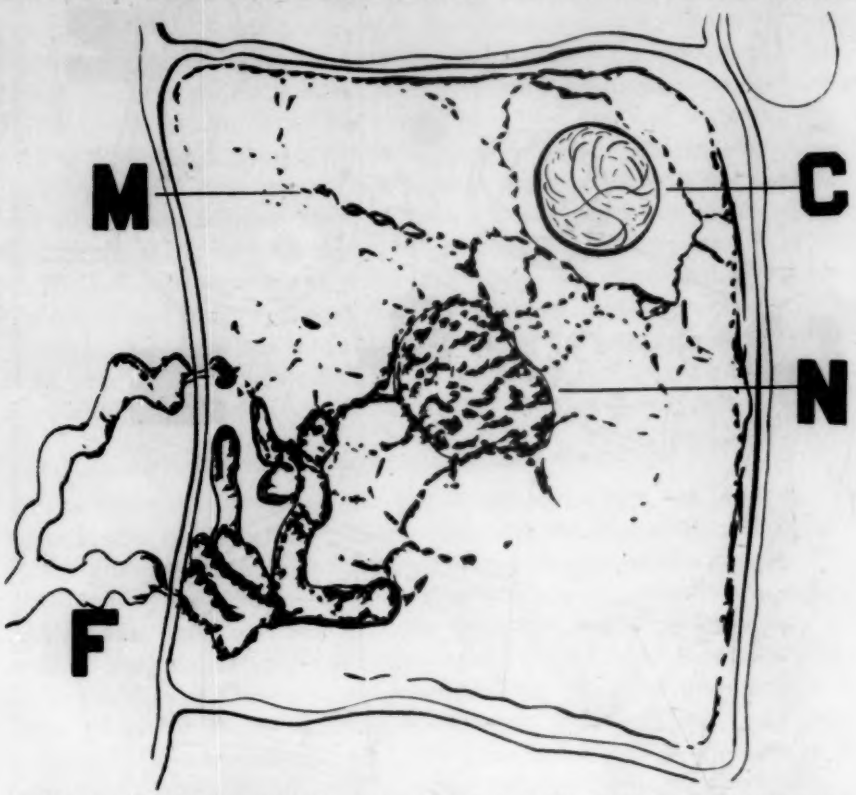

FIG. 2. A cell from stalk of sugar cane penetrated by hyphae, F, of *C. falcatum* fixed in HELLY fluid and stained with haematoxylin. The nucleus, N, at the center of the cell shows numerous prochromosomes; mitochondria, M, are lined up between a number of vacuoles. The segregated vacuole in the upper right corner contains a large coacervate, C, outlined by the deeply stained phospholipoid envelope.

compounds can be dissolved out by proper solvents (for instance tetra ethylene glycol or methyl ethers), leaving the envelope intact as an empty shell.

Whereas coacervates are constantly present in nodes of young canes affected by chlorotic streak, they generally do not occur in homologous tissues of young, actively growing, healthy canes, but they make their appearance later, toward the end of the growth cycle, about the time for harvest. They also appear in cells affected by various diseases besides chlorotic streak, and

they can be made to appear at will, following injection of the stalks by dilute solutions of agents acting as inhibitors of some respiratory systems, for instance salicylaldoxime (10 to 100 p.p.m.) or sodium xanthogenate (100 p.p.m.).

Coacervates similar to those observed in chlorotic streak also occur in cells of cane infected by *Colletotrichum falcatum*, the pathogenic fungus responsible for the Red Rot disease (fig. 2). The disease is characterized by a discoloration due to anthocyanol formation in the vacuolar solution of cells adjoining the area of infection. Conidia of the fungus travel upwards and downwards along the pitted vessels from the point of inoculation, having a tendency to lodge and germinate a short distance below the nodes. The germ tubes which grow through pits into the adjoining slender, elongated parenchyma cells cause the vacuolar solutions to be dispersed into a number of vacuoles possessing high oxidase activity, and soon producing the above-mentioned red pigment. Some of this oxidized, polymerized material oozes from the vacuoles through pits in the wall into tyloses. That reaction efficiently blocks any further progress of the germ tubes in resistant canes. In susceptible canes, however, the germ tubes grow as hyphae from one parenchyma cell to the next, or in the intercellular spaces, inducing noncompensated respiration in cells as much as five or six rows distant, as evidenced by the oxidation of paraphenylene diamine hydrochloride to the red quinoid derivative (Würster red) in the vacuolar solutions. The red anthocyanol pigment formed in the parenchyma cells of susceptible cane oozes from the vacuoles into the intercellular spaces and coacervates may appear in some vacuoles. In Red Rot tissues we therefore witness both the external secretion of vacuolar material, even to the intercellular spaces, and also that peculiar type of "internal secretion" which results in coacervate formation.

Cell inclusions in the vine disease, now called "Pierce's disease," were observed by VIALA and SAUVAGEAU in 1892 (**15**) and then misinterpreted as plasmodia of a Myxomycete. DUCOMET (**5**) abandoned the erroneous idea of plasmodial parasites and gave a cytochemically correct interpretation of the small and large spherical bodies in the vacuolar sap, opening the way to the recognition of the prevalence in other plants of those phenolic or tannic compounds which may become conspicuously coacervated or otherwise polymerized (**12**). Pierce's disease is characterized physiologically by the failure of internodes to elongate and cytochemically by coacervation of phenolic compounds. A similar correlation between dwarfed shoots and coacervation was demonstrated as the result of zinc deficiency in apricots and walnuts (REED and DUFRENOY) (**13**).

Cytochemical examination of grapes experimentally infected in the greenhouses of the University of California by Dr. J. H. FREITAG demonstrates the prevalence of coacervates in the perivascular cells of the stems. A longitudinal section after proper fixation in the HELLY's killing fluid and staining with haematoxylin, showed distorted nuclei (some with amyloplasts

closely appressed) and one or several coacervates sharply outlined. In contiguous cells the phenolic material was often flocculated in the vacuole, and many times could be distinguished as a mass of small spherical drops. In other cases the vacuolar space was almost filled with a diffuse material giving the chromaffinic reaction.

Cytochemical reaction

Coacervates in the vacuolar solution appear as features in a three phase system. The inner phase may be defined as a "gelatinous hydrate" of phenolic compounds and the outer phase as the depleted vacuolar solution. The intermediate phase is made of amphoteric phospholipids. The identification of coacervates therefore calls for the cytochemical localization of the phenolic compounds and of the phospholipids.

Phenolic compounds

The cytochemical reactions may range from those indicative of some active C–OH group or dienol HO–C–C–OH or paraphenol, to those of highest specificity for some definite configuration of the ring structure or active groups.

The reactions resulting in the formation of bright azo-dyes have been shown by Lison to grade from the general localization of phenolic compounds to the specification of definite compounds, according to the cytochemical technique applied. The diazo-reaction, which Swaminathan (**14**) used for the quantitative estimation of vitamin B_6 may also be used for the detection of pyridoxin in coacervates, or in sections of fresh plant material. A convergent line of evidence as to the presence of pyridoxin in coacervates may be obtained by simply immersing freehand sections of tissues in a suspension of 2.6 dichloroquinone chloroimide buffered at pH 8.8 with sodium borate (**6**).

Depleted vacuolar solutions

The vacuolar solution in which coacervates are floating is evidently depleted in dispersed materials which would respond to vital staining, and concomitantly depleted in PO_4 ions, since the phenolic compounds apt to absorb the vital dyes have become coacervated in the inner phase of the coacervate, and the ionic PO_4 has entered into combination with lipids to form the third or intermediate phase of the auto-complex coacervate.

Sections of tissues at the site of non-compensated respiration may show cells where coacervates are sharply outlined from the otherwise empty vacuole, in contrast to the neighboring cells, where phenols have not been coacervated as yet, and whose vacuolar solution stains as a whole with vital dyes, or responds to the chromaffin reaction.

Third (intermediate) phase

This phase may be identified as to either of its constituents: The phosphorus being responsible for the formation of molybdenum blue with

molybdenum reagent, and the fatty materials staining solid black a few minutes after the section has been immersed in the hydrated methylal solution of Sudan Black (7). The intermediate phase represents a solid physical structure, which persists as an empty shell after the phenolic contents have been dissolved out by proper solvents such as polyethylene glycol or by methyl ethers. In healthy cells the PO_4 ions from the vacuolar solution are used almost as fast as they become available for the building up of nucleic acids and other energy-rich phosphorus compounds through esterification. In hypoplastic cells, coacervation ties up phosphorus in the solid auto-complex structure, thus making it unavailable for phosphorylation of carbohydrates in cell metabolism, which may account for the dwarfed shoots characteristic of rosette of fruit trees or the "Court Noué" of vines.

It remains for future investigations to show how the activity of the enzymic systems may be influenced by the alterations in equilibria due to the formation of auto-complex coacervates in cell vacuoles.

Summary

1. The study of coacervation in hypoplastic cells demonstrates some important correlations with the respiratory activities in the vacuoles.

2. Auto-complex coacervates appear as refringent spherical bodies in the vacuoles of hypoplastic cells affected by virus, or parasitized by certain fungi, or deficiency of some essential microelement. They have also been induced in tissues treated with compounds tending to block some component of the cell respiratory system.

3. The data presented show that these refringent spherical bodies result from the coacervation of phenolic compounds from the vacuolar solution concomitant with the adsorption of phosphatides at the interphase between the coacervated compounds and the water phase of the vacuole. Both physico-chemical phenomena are related to a shift of the respiratory systems toward a higher rH.

4. Coacervation is one of the several ways whereby a phase originally homogeneous may become differentiated into several. Coacervation of phenolic materials within the vacuole does not imply any change of cytoplasmic permeability (although the same disorders in cell respiration which induce coacervation may enhance permeability).

A portion of the work herein reported was accomplished while the senior author was an exchange professor at the University of Louisiana.

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CONCERNING THE FUNCTION OF RUBBER HYDROCARBON (CAOUTCHOUC) IN THE GUAYULE PLANT, *PARTHENIUM ARGENTATUM* A. GRAY[1]

HAMILTON P. TRAUB

(WITH FOUR FIGURES)

LLOYD (**11, 12**), who originally believed that rubber hydrocarbon (caoutchouc) in the guayule plant occurred in the parenchymatous cells as solid particles, stated that "rubber appears to have no physiological function in the guayule plant"; but this viewpoint was questioned by SPENCE (**21**) on the basis of work with oxydases in Hevea latex. Later LLOYD (**13**) demonstrated that the rubber in the parenchymatous cells of guayule "occurs as a colloidal suspension, precisely as in the latex of Hevea," and this led him to modify his viewpoint for he realized the possibility that rubber in colloidal suspension could be attacked by enzymes. In support of this hypothesis could be cited the work of FAIVRE (**2, 3, 4**), with *Tragopogon porrifolius, Morus alba,* and a *Ficus sp.*, who varied the conditions, including absence of carbon dioxide, under which they were grown, and observed impoverishment of the latex; and the experiment of SPENCE (**21**) who reports that young *Ficus elastica* "grown in an atmosphere and soil free of carbon dioxide, gradually drew on their milk which became nothing more than water after a few weeks' time." However, LLOYD (**13**) insisted that proof was required for the hypothesis that the rubber hydrocarbon in guayule is actually a reserve substance and is made use of in metabolism.

The results of SPENCE and MCCALLUM (**22**) showed a loss in grams of rubber per plant during conditions that favored utilization of carbohydrate reserves. However, observations during the course of experiments from 1942 to 1944 showed no such loss of rubber occurring in guayule plants under similar conditions. It was thought possible because of the great variability of seedling guayule plants that the results of SPENCE and MCCALLUM (**22**) may have been vitiated by failure to adequately replicate the treatments in order to eliminate the possibility of the differences being nothing more than chance variation. Since this subject is very important not only from the theoretical standpoint of plant physiology but also in connection with guayule rubber production, the work here reported was undertaken to test the conclusions of previous workers.

Methods

Guayule nursery seedlings were grown in gravel culture in the greenhouse under three levels of carbon assimilation:

Treatment I. No leaves removed.

[1] Contribution from the Emergency Guayule Research Project, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

Treatment II. All leaves removed at the beginning of the experiment.

Treatment III. All leaves removed at the beginning of the experiment, and new top growth at intervals after growth began.

The very low level of carbon assimilation in treatment III was calculated to exhaust the carbohydrate reserves in the plant in order to test the hypothesis that rubber hydrocarbon (caoutchouc) may be used as a food.

The 1500 plants required for the experiment were selected for uniformity from more than 20,000 field run plants that had been seeded in June, 1943, on Chualar loam soil at the Alisal Nursery of the Emergency Rubber Project, Salinas, Calif., and were therefore 16 months old when the plots were set up in October, 1944. The roots were cut uniformly to 6 inches. In physiological studies, the results are usually expressed on the basis of total dry weight which is a changing basis when the developing plant is involved. MASON and MASKELL (**14**) have suggested the residual dry weight as an improvement for it is less subject to change. In the present experiment, the rubber, resins and carbohydrate results are expressed on the basis of constituents, in grams, in relatively constant regions: The original (a) 6-inch (15.24 cm.) root with crown diameter of 6.4 ± 0.066 mm., and (b) stems with 5 ± 0.12 branches; height 16.6 ± 0.206 cm. In addition, the new roots, and new growth were analyzed for rubber, resins, and carbohydrates.

The plants were grown in gravel culture in ½-gal. glazed crocks provided with a hole for drainage on one side near the bottom. Three plants were grown in each crock, and five such crocks with plants, 15 plants in all, constituted the unit plots for sampling purposes. Each treatment for each of the three sampling dates was randomly replicated ten times. The plants were given Hoagland's nutrient solution No. 1, supplemented with boron, manganese, zinc, copper, molybdenum and iron as recommended by HOAGLAND and ARNON (**9**) and flushed out with tap water on alternate days. The night temperature in the greenhouse was kept at approximately 75° F., and the day temperature ranged between 80° and 90° F.

All of the plots were sampled at the beginning of the experiment on Oct. 21, 1944, and again on Dec. 19, 1944, and Jan. 19, 1945. The fourth sampling for treatment III was made on Feb. 15, 1945.

The plants, with ball of gravel containing any new roots, were carefully removed by tipping the top of the crocks downward, and the new roots with gravel were held in a tub of water thus allowing the loose gravel to sink to the bottom. When all 15 plants constituting the particular unit plot had been treated in this manner, the new root portions left behind in the tub of water were recovered according to the method described by TRAUB (**25**) and added to the new root fraction of the plot. At the first sampling, the plants were separated into three portions: tap roots, branches, and leaves. Thereafter two portions were added; new roots and new top growth.

The various fractions were separately dried in a mechanical convection oven at 65° C., for 48 hours, and after weighing for the determination of total dry weight, were finely ground according to the procedure described

by TRAUB (**24**) for rubber, resins and carbohydrate analyses. It should be noted that the new roots fraction still contained any adhering gravel after drying, but this was removed before the tissue was ground, according to the procedure described by TRAUB (**25**).

After the experiment was started, new top growth was removed from plants of treatment III at intervals (Nov. 20, Dec. 5, Dec. 19, Jan. 9, Jan. 19, and Feb. 15) in order to keep the assimilation of carbon at a relatively low level. The new top growth removed at intervals between regular sampling dates was included with the dry weight and the tissues analyzed for the various constituents at the regular sampling dates.

Rubber and resins were determined according to the photometric procedure of TRAUB (**24**). Total water soluble carbohydrates, free sugars, levulins, inulin, and the relative amounts of 89 per cent. ethanol insoluble and 89 per cent. ethanol soluble levulins were analyzed according to the procedure described by TRAUB and SLATTERY (**26**), and pentosans according to the method of MCRARY and SLATTERY (**15**). The data have been subjected to appropriate statistical analysis, in most cases by the use of FISHER'S analysis of variance (**7, 5, 6, 20**).

Data

GROWTH RESPONSES

Plants not defoliated (treatment I) showed a lag in growth resumption after the experiment was set up in the greenhouse. After two months, on

FIG. 1. Growth responses of guayule plants as affected by three levels of carbon assimilation: 1. Not defoliated; 2. Once Defoliated; 3. Defoliated and new growth repeatedly removed. Upper, at end of period, Oct. 21 to Dec. 19, 1944; and lower, at end of period, Dec. 20, 1944, to Jan. 19, 1945.

Dec. 19, 1944, only 78.6 per cent. of the plants under treatment I had sprouted. In contrast, similar plants when defoliated (treatments II and III) sprouted promptly after planting. These results are in harmony with those reported by SMITH (**18, 19**) and ERICKSON (**1**).

The plants in treatment I, with no leaf removal, soon appeared somewhat shriveled and apparently were suffering from excessive moisture loss through transpiration, an effect similar to that reported by ERICKSON (**1**). By Jan. 19, all of the plants in treatment I had resumed growth, but there was variation in degree of development as shown in figure 1. Such variation in growth responses was apparently due to the injurious effect on plant tissues when there was a moisture deficit in the plants for a time after the experiment was started in October.

TABLE I

EFFECT OF DEFOLIATION ON MEAN DRY WEIGHT, IN GRAMS PER PLOT, OF PLANT ORGANS AS INDICATED; GUAYULE PLANTS GROWN IN GREENHOUSE, SALINAS, CALIFORNIA, 1944–1945

PLANT ORGAN	SAMPLING DATES	WEIGHT PER PLOT†			DIFFERENCE REQUIRED FOR SIGNIFICANCE			
		NOT DEFOLIATED	ONCE DEFOLIATED	REPEATEDLY DEFOLIATED	MEANS*		INTERACTIONS*	
					0.05	0.01	0.05	0.01
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Original leaves and new top growth‡	Oct. 21	15.67	15.72	15.33	1.64	2.20	2.32	3.11
	Dec. 19	18.70 (+ 3.03)§	13.47	6.45				
	Jan. 19	32.36 (+16.69)	30.25	9.61				
Original stem and branches	Oct. 21	47.38	45.62	47.09	2.52	3.56	3.38	4.78
	Dec. 19	45.25	39.84	38.33				
	Jan. 19	40.28	40.93	35.89				
Original 6″ roots	Oct. 21	24.72	24.44	24.43	1.42	1.91	2.01	2.70
	Dec. 19	23.80	24.52	21.74				
	Jan. 19	24.74	25.23	20.10				
New roots	Dec. 19	0.47	1.72	0.72	0.55	0.75	0.78	1.06
	Jan. 19	5.00	8.35	1.24				

* If values for treatments differ by more than the figure given in these columns, the odds are greater than 19 : 1 (0.05) or 99 : 1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten randomized 15-plant plots.

‡ Original leaves at beginning of experiments, and total original leaves plus new growth, stem and leaves in case of plants not defoliated and total new growth, stem and leaves, in case of defoliated plants, up to the dates indicated.

§ Values in parentheses indicate gain or loss over original dry weights of leaves.

In the case of plants defoliated at the beginning of the experiment only (treatment II), growth resumption was rapid and total growth of tops and roots was superior to plants of the other two treatments (I and III) as shown in figure 1.

Plants of treatment III, defoliated at the beginning of the experiment, followed by removal of new top growth at intervals thereafter, started out exactly like the plants in treatment II, but after each defoliation there was

a lag in growth followed by renewed sprouting and growth. The total effect of the repeated removal of new top growth was distinctly shown in the relatively poor root development as indicated in figure 1. In a few cases, toward the end of the experiment (after Jan. 19), the tips of the branches died back for ¼ to ½ inch, but such dying back did not affect the rubber content as will be shown below.

The dry weight data on the basis of grams per plot are summarized in table I, together with analyses of variance.

In connection with the subject of changes in total dry weight it will be of interest to consider the amount of dry matter lost to the guayule plant,

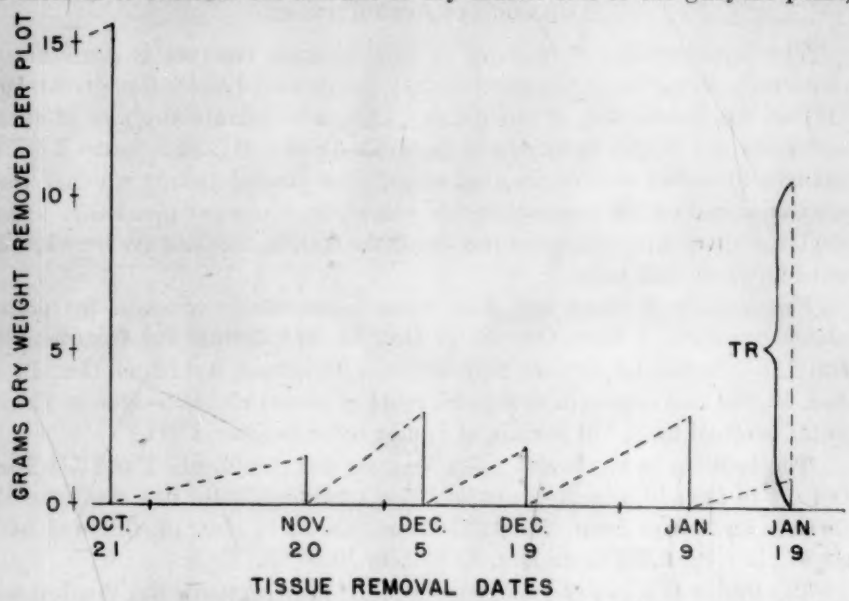

FIG. 2. Amount of dry weight, in grams, removed per plot from guayule plants in treatment III by repeated defoliations from Oct. 21, 1944, to Jan. 19, 1945. Solid vertical lines represent grams dry weight removed per plot on harvesting dates indicated; line enclosed by bracket, TR, represents total new top growth removed per plot after initial defoliation on Oct. 21.

in treatment III, by the repeated removal of new growth. The data are shown graphically in figure 2. This shows that 15 grams of dry matter per plot were removed at the beginning of the experiment, and that during the following three and a half months, 10 grams of dry matter were lost to these plants due to removal of new growth. In contrast, the increase in dry matter per plot due to new growth, for treatments I and II, was not lost to the plants. From the standpoint of total grams of dry matter produced irrespective of its removal, plants of treatment I, not defoliated, laid down approximately half as much dry weight per plot (16.5 gm.) as plants of treatment II, once defoliated (30.3 gm.); and plants of treatment III, repeatedly defoliated, produced only about one-third as much (9.6 gm.) per plot.

The original stems and branches lost dry weight in all cases, but the loss in plants of treatment III, repeatedly defoliated (−11 gm. per plot) was greatest. Roots showed a loss in dry weight only for plants of treatment III, repeatedly defoliated (−4.7 gm.). The marked losses of dry weight per plot in stems and roots for this severe treatment demonstrate the effectiveness of the technique of repeated removal of new top growth for starving plants.

The increase in dry weight per plot due to new root growth was greatest for plants of treatment II, once defoliated (8.4 gm.), and lowest for plants of treatment III, repeatedly defoliated (1.34 gm.).

Changes in carbohydrates

The consideration of changes in carbohydrate reserves is particularly important in evaluating the effect of very low carbon assimilation (treatment III) on the functioning of the plant. The carbohydrate analyses of stems and roots are briefly summarized in tables II and III, and figure 3. The changes in carbohydrates, in most cases, show similar trends whether they are considered on the percentage dry weight, or grams per plot basis. Since the latter gives a truer approximation of the trends, the data are here briefly considered on that basis.

Free sugars of stems and roots remain practically constant for plants under treatment I from Oct. 21 to Dec. 19, but decline for treatment II and III. In the latter case they decrease to almost 0. From Dec. 19 to Jan. 19, the free sugars in stems and roots of plants under treatment II rise to the original level, but remain at almost 0 for treatment III.

The levulins in stems and roots decrease for treatments I and III from Oct. 21 to Dec. 19, reaching almost 0 for treatment III. For treatment II there is an abrupt drop ($P = 0.01$) from Oct. 21 to Dec. 19, followed by a slight rise ($P = 0.05$) from Dec. 19 to Jan. 19.

The inulin of stems and roots declines for all treatments but the decrease is at a slower rate for treatment I for the low is reached for treatments II and III by Dec. 19 whereas a similar low is not reached for plants of treatment I until Jan. 19. Again the drop for treatment I is almost to 0.

The changes in total water-soluble carbohydrates for the three treatments show the same tendencies as those of the levulins which make up the greatest proportion of the whole.

Similarly the 89 per cent. ethanol soluble levulins showed the same tendencies as the levulins, but the 89 per cent. ethanol insoluble levulins followed trends similar to those shown by the inulin contents.

The pentosans of stems show a decline for all treatments from Oct. 21 to Jan. 19, but the decrease is greatest for plants under treatment III. The pentosan content of roots did not change perceptibly from Oct. 21 to Jan. 19, except for a decrease ($P = 0.01$) for plants under treatment III by Jan. 19.

These results show conclusively that the plants under treatment III, repeatedly defoliated, were so effectively starved on account of the very low level of carbon assimilation that the water-soluble carbohydrate content—

TABLE II

EFFECT OF DEFOLIATION ON MEAN PERCENTAGE CARBOHYDRATES, OF PLANT ORGANS AS INDICATED; GUAYULE PLANTS GROWN IN GREENHOUSE, SALINAS, CALIFORNIA, 1944–1945

PLANT ORGAN	SAMPLING DATES	PERCENTAGE DRY WEIGHT			DIFFERENCE REQUIRED FOR SIGNIFICANCE			
		NOT DEFOLIATED	ONCE DEFOLIATED	REPEATEDLY DEFOLIATED	MEANS*		INTERACTIONS*	
					0.05	0.01	0.05	0.01
		%	%	%	%	%	%	%
FREE SUGARS								
Stems†	Oct. 21	0.54	0.53	0.55	0.06	0.08	0.08	0.11
	Dec. 19	0.65	0.34	0.20				
	Jan. 19	0.57	0.59	0.20				
Roots†	Oct. 21	0.58	0.57	0.59	0.08	0.11	0.11	0.16
	Dec. 19	0.57	0.43	0.25				
	Jan. 19	0.53	0.62	0.14				
LEVULINS								
Stems	Oct. 21	11.35	11.39	11.28	0.67	0.89	0.95	1.26
	Dec. 19	5.58	0.93	0.52				
	Jan. 19	2.44	1.70	0.19				
Roots	Oct. 21	14.51	14.75	14.64	0.73	0.98	1.03	1.39
	Dec. 19	8.14	2.19	1.07				
	Jan. 19	5.16	4.41	0.30				
INULIN								
Stems	Oct. 21	1.28	1.19	1.22	0.33	0.44	0.47	0.62
	Dec. 19	0.78	0.20	0.22				
	Jan. 19	0.70	0.30	0.21				
Roots	Oct. 21	2.13	2.20	2.21	0.47	0.64	0.66	0.90
	Dec. 19	1.40	0.52	0.48				
	Jan. 19	0.77	0.45	0.13				
TOTAL WATER-SOLUBLE CARBOHYDRATES								
Stems	Oct. 21	13.18	13.11	13.16	0.77	1.04	1.09	1.47
	Dec. 19	7.00	1.48	0.94				
	Jan. 19	3.71	2.60	0.61				
Roots	Oct. 21	17.21	17.52	17.56	0.75	1.01	1.06	1.48
	Dec. 19	10.21	3.14	1.79				
	Jan. 19	6.47	5.48	0.56				
89 PER CENT. ETHANOL SOLUBLE LEVULINS								
Stems	Oct. 21	3.00	2.73	2.87	0.70	0.94	0.99	1.33
	Dec. 19	1.49	0.67	0.36				
	Jan. 19	1.42	1.69	0.22				
Roots	Oct. 21	3.16	3.41	3.29	0.69	0.92	0.98	1.30
	Dec. 19	2.86	1.34	0.61				
	Jan. 19	3.14	3.89	0.22				

TABLE II.—(*Continued*)

Plant organ	Sampling dates	Percentage dry weight			Difference required for significance			
		Not defoliated	Once defoliated	Repeatedly defoliated	Means*		Interactions*	
					0.05	0.01	0.05	0.01
		%	%	%	%	%	%	%
80 per cent. ethanol insoluble levulins								
Stems	Oct. 21	9.64	9.75	9.84	0.71	0.95	1.00	1.34
	Dec. 19	4.87	0.47	0.38				
	Jan. 19	1.72	0.32	0.18				
Roots	Oct. 21	13.48	13.53	13.68	0.66	0.88	0.93	1.24
	Dec. 19	6.79	1.37	0.93				
	Jan. 19	2.75	0.84	0.26				
Pentosans								
Stems	Oct. 21	2.52	2.52	2.50	0.08	0.10	0.11	0.16
	Dec. 19	2.19	2.10	2.05				
	Jan. 19	2.21	2.24	1.79				
Roots	Oct. 21	1.23	1.23	1.24	0.08	0.11	0.11	0.16
	Dec. 19	1.22	1.22	1.24				
	Jan. 19	1.32	1.39	1.15				

* If values for treatments differ by more than the figures given in these columns, the odds are greater than 19 : 1 (0.05) or 99 : 1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten randomized 15-plant plots.

free sugars, levulins and inulin—declined almost to 0 from Oct. 21 to Jan. 19. Even the pentosan reserves, not so readily available, were drawn upon due to the starved condition of the plants for the pentosan content of stems declined ($P = 0.01$) from 2.50 to 1.79 per cent., or from 1.18 to 0.64 grams per plot. Similarly the pentosans in roots declined ($P = 0.05$) from 1.24 to 1.15 per cent., or ($P = 0.01$) from 0.30 to 0.23 grams per plot. This is also the first concrete demonstration that pentosans in guayule, particularly in the stems, function as food reserves.

In figure 3 are shown graphically any gains and losses in total dry weight over the original weights, and the carbohydrate reserves (free sugars, levulins, inulin and pentosans) considered above. In the case of guayule stems the losses in dry weight for treatments I and III are greater than the losses in the reserve carbohydrates determined. In the roots there is a slight gain in dry weight for treatment II, no net loss for treatment I, and a decided loss in dry weight for treatment III. The losses in reserve carbohydrates for treatments I and II are not counter balanced by similar changes in dry weight as is the case for treatment III.

This suggests the possibility that in the stems still other compounds in the plant, such as pectins, may serve as carbon reserves. It is apparently necessary for the physiologist to go farther than the usual routine analyses in order to arrive at an adequate understanding of plant functioning.

TABLE III

EFFECT OF DEFOLIATION ON MEAN CARBOHYDRATE CONTENT, IN GRAMS PER PLOT, OF PLANT ORGANS AS INDICATED; GUAYULE PLANTS GROWN IN GREENHOUSE, SALINAS, CALIFORNIA, 1944–1945

PLANT ORGAN	SAMPLING DATES	WEIGHT PER PLOT			DIFFERENCE REQUIRED FOR SIGNIFICANCE			
		NOT DEFOLIATED	ONCE DEFOLIATED	REPEATEDLY DEFOLIATED	MEANS*		INTERACTIONS*	
					0.05	0.01	0.05	0.01
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
FREE SUGARS								
Stems†	Oct. 21	0.25	0.24	0.26	0.03	0.04	0.04	0.06
	Dec. 19	0.29	0.14	0.08				
	Jan. 19	0.23	0.24	0.07				
Roots†	Oct. 21	0.15	0.14	0.15	0.02	0.03	0.03	0.04
	Dec. 19	0.14	0.11	0.06				
	Jan. 19	0.13	0.16	0.03				
LEVULINS								
Stems	Oct. 21	5.38	5.21	5.31	0.42	0.57	0.59	0.81
	Dec. 19	2.53	0.37	0.20				
	Jan. 19	0.98	0.70	0.07				
Roots	Oct. 21	3.58	3.61	3.61	0.22	0.29	0.31	0.41
	Dec. 19	1.91	0.54	0.24				
	Jan. 19	1.28	1.11	0.06				
INULIN								
Stems	Oct. 21	0.60	0.54	0.57	0.15	0.20	0.21	0.28
	Dec. 19	0.36	0.08	0.08				
	Jan. 19	0.28	0.13	0.08				
Roots	Oct. 21	0.53	0.54	0.54	0.12	0.16	0.17	0.23
	Dec. 19	0.34	0.13	0.10				
	Jan. 19	0.19	0.11	0.03				
TOTAL WATER-SOLUBLE CARBOHYDRATES								
Stems	Oct. 21	6.24	5.90	6.19	0.45	0.60	0.64	0.85
	Dec. 19	3.18	0.59	0.36				
	Jan. 19	1.49	1.07	0.22				
Roots	Oct. 21	4.26	4.28	4.29	0.23	0.31	0.33	0.44
	Dec. 19	2.39	0.78	0.40				
	Jan. 19	1.60	1.38	0.11				
89 PER CENT. ETHANOL SOLUBLE LEVULINS								
Stems	Oct. 21	1.41	1.25	1.35	0.33	0.44	0.47	0.62
	Dec. 19	0.68	0.27	0.14				
	Jan. 19	0.58	0.70	0.08				
Roots	Oct. 21	0.78	0.83	0.80	0.17	0.23	0.24	0.33
	Dec. 19	0.68	0.33	0.14				
	Jan. 19	0.78	0.98	0.04				

TABLE III.—(*Continued*)

Plant organ	Sampling dates	Weight per plot			Difference required for significance			
		Not defoliated	Once defoliated	Repeatedly defoliated	Means*		Interactions*	
					0.05	0.01	0.05	0.01
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
89 per cent. ethanol insoluble levulins								
Stems	Oct. 21	4.57	4.46	4.64	0.43	0.58	0.61	0.82
	Dec. 19	2.28	0.19	0.15				
	Jan. 19	0.68	0.13	0.07				
Roots	Oct. 21	3.33	3.31	3.34	0.20	0.27	0.28	0.38
	Dec. 19	1.61	0.34	0.21				
	Jan. 19	0.68	0.22	0.04				
Pentosans								
Stems	Oct. 21	1.20	1.15	1.18	0.08	0.11	0.11	0.16
	Dec. 19	0.99	0.84	0.79				
	Jan. 19	0.89	0.92	0.64				
Roots	Oct. 21	0.30	0.30	0.30	0.03	0.03	0.04	0.04
	Dec. 19	0.29	0.30	0.27				
	Jan. 19	0.33	0.35	0.23				

* If values for treatments differ by more than the figure given in these columns, the odds are greater than 19 : 1 (0.05) or 99 : 1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten randomized 15-plant plots.

Changes in rubber content

The changes in rubber content are summarized in tables IV and V. In the former, the data are expressed in percentage of total dry weight, and in the latter in grams of rubber present in the regions, of new growth, original stems and branches, original 6-inch roots, and new roots.

In the case of original leaves and new top growth, by Dec. 19, on a percentage basis, a loss of 0.1 per cent ($P = 0.05$) in rubber is indicated for treatment I, but on the basis of grams of rubber for the entire region no significant change is shown (+0.001 gm.). At the Jan. 19 sampling significant increases are indicated ($P = 0.01$) on both the percentage dry weight and grams per region basis. Plants defoliated at the beginning of the experiment, treatments II and III, accumulated rubber, by Dec. 19, in the original leaves and new growth, and showed no increase ($P = 0.01$) from treatment III, but a decided increase, from 0.05 gm. per plot by Dec. 19 to 0.16 grams on Jan. 19. It should be noted that the rubber content in this region either remained stationary or increased.

In the case of the original stems and branches, initial increases ($P = 0.01$), on the percentage and regional bases, from Oct. 21 to Dec. 19 are shown for all treatments. From Dec. 19 to Jan. 19, a significant increase (0.01), on the percentage basis, for treatment I is shown, but no increases for treat-

ments II and III. However, on the grams per plot basis, no significant changes are indicated.

In the case of the original 6-inch roots, on the percentage basis, significant increases ($P = 0.01$) in rubber content are indicated from Oct. 21 to Dec. 19, and from Dec. 19 to Jan. 19 for all treatments, excepting the period Oct. 21 to Dec. 19, for treatment II, in which case an increase is indicated only on the $P = 0.05$ level. On the grams per plot basis, however, the situation is somewhat different. From Oct. 21 to Dec. 21, significant increases

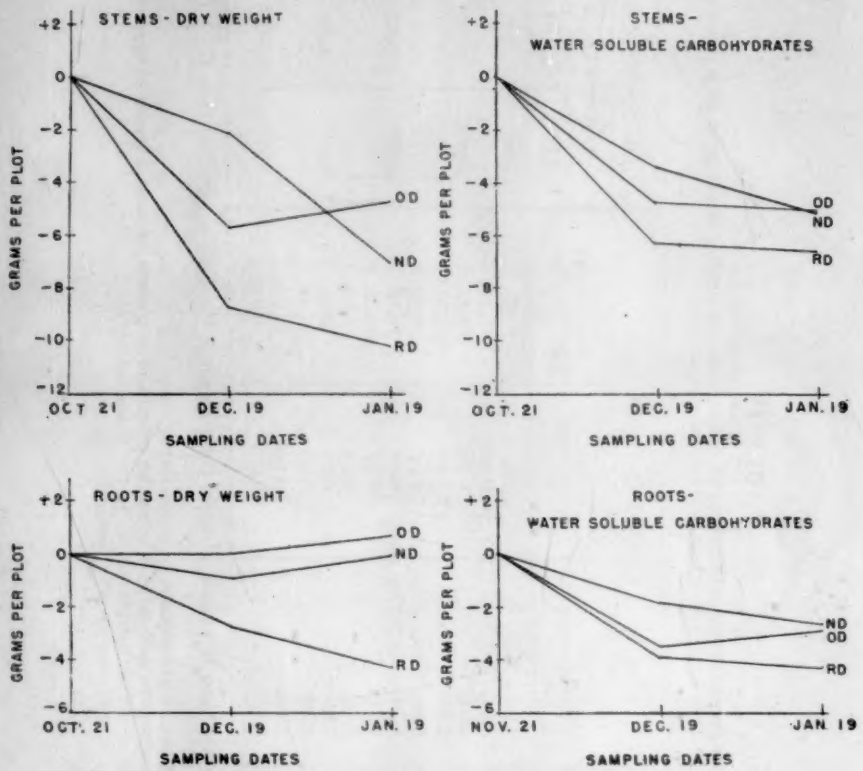

FIG. 3. Changes in dry matter and water-soluble carbohydrates (free sugars, levulins and inulin) of guayule plants, on grams per plot basis; treatments I (ND) not defoliated, II (OD) once defoliated, and III (RD) repeatedly defoliated.

($P = 0.01$) are indicated only for treatments I and III and not for the rapidly growing plants in treatment II. From Dec. 19 to Jan. 19, however, the tendencies are reversed, for there is then a significant increase ($P = 0.01$) for the normally developing plants of treatment II, and none for the retarded plants of treatments I and III.

A final harvest of plants under treatment III was made on Feb. 15. The percentages of rubber on this date for the original stems and roots were 10.86 and 11.08, respectively; and the corresponding grams of rubber per plot were 4.08 and 2.20, respectively, thus showing no significant changes in this constituent from Jan. 19 to Feb. 15.

TABLE IV

EFFECT OF DEFOLIATION ON MEAN PERCENTAGE RUBBER IN PLANT ORGANS AS INDICATED; GUAYULE PLANTS GROWN IN GREENHOUSE, SALINAS, CALIFORNIA, 1944–1945

PLANT ORGAN	SAMPLING DATES	PERCENTAGE RUBBER†			DIFFERENCE REQUIRED FOR SIGNIFICANCE			
		NOT DEFOLIATED	ONCE DEFOLIATED	REPEATEDLY DEFOLIATED	MEANS*		INTERACTIONS*	
					0.05	0.01	0.05	0.01
		%	%	%	*gm.*	*gm.*	*gm.*	*gm.*
Original leaves and new top growth‡	Oct. 21	0.641	0.635	0.639	0.076	0.102	0.107	0.144
	Dec. 19	0.541 (−0.100)§	0.398	0.322				
	Jan. 19	0.890 (+0.249)	0.553	0.346				
Original stem and branches	Oct. 21	6.98	6.96	6.90	0.61	0.82	0.86	1.16
	Dec. 19	8.70	9.86	10.76				
	Jan. 19	9.81	10.22	11.36				
Original 6″ roots	Oct. 21	5.38	5.54	5.29	0.57	0.76	0.81	1.07
	Dec. 19	7.63	6.13	9.27				
	Jan. 19	8.52	8.25	10.44				
New roots	Dec. 19	0.158	0.186	0.194	0.057	0.078	.....	.....
	Jan. 19	0.134	0.237	0.143				

* If values for treatments differ by more than the figure given in these columns, the odds are greater than 19 : 1 (0.05) or 99 : 1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten 15-plant plots.

‡ Original leaves at beginning of experiments, and total original leaves plus new growth, stem and leaves in case of plants not defoliated and total new growth, stem and leaves, in case of defoliated plants, up to the dates indicated.

§ Values in parentheses indicate gain or loss over original percentages.

TABLE V

EFFECT OF DEFOLIATION ON MEAN AMOUNT OF RUBBER IN GRAMS PER PLOT, IN PLANT ORGANS AS INDICATED; GUAYULE PLANTS GROWN IN GREENHOUSE, SALINAS, CALIFORNIA, 1944–1945

PLANT ORGAN	SAMPLING DATES	AMOUNT RUBBER PER PLOT†			DIFFERENCE REQUIRED FOR SIGNIFICANCE			
					MEANS*		INTERACTIONS*	
		NOT DEFOLIATED	ONCE DEFOLIATED	REPEATEDLY DEFOLIATED	0.05	0.01	0.05	0.01
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Original leaves and new top growth‡	Oct. 21	0.100	0.100	0.099	0.016	0.022	0.023	0.031
	Dec. 19	0.101 (+0.001)§	0.054	0.021				
	Jan. 19	0.285 (+0.185)	0.167	0.033				
Original stem and branches	Oct. 21	3.32	3.19	3.25	0.34	0.45	.....	.....
	Dec. 19	3.92	3.93	4.12				
	Jan. 19	3.94	4.13	4.08				
Original 6″ roots	Oct. 21	1.33	1.36	1.29	0.19	0.25	0.27	0.35
	Dec. 19	1.82	1.50	2.01				
	Jan. 19	2.11	2.08	2.10				
New roots	Dec. 19	0.0007	0.0032	0.0014	0.0035	0.0046	0.0049	0.0067
	Jan. 19	0.0063	0.0196	0.0018				

* If values for treatments differ by more than the figure given in these columns, the odds are greater than 19 : 1 (0.05) or 99 : 1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten 15-plant plots.

‡ Original leaves at beginning of experiments, and total original leaves plus new growth, stem and leaves in case of plants not defoliated and total new growth, stem and leaves, in case of defoliated plants, up to the dates indicated.

§ Values in parentheses indicate gain or loss over original amounts present.

By Dec. 19 new roots had formed on all plants of treatments II and III, and on most of the plants in treatment I. From Dec. 19 to Jan. 19 no significant change in new roots could be shown even at the 0.05 level on a percentage of dry weight basis. On the basis of grams per plot significant increases ($P = 0.01$) are shown for plants of treatments I and II, but none for treatment III.

These data show that the use of the percentage of dry weight basis in expressing the experimental results gave a somewhat different picture from that presented when results were expressed as grams present in a region of the plant. When results were expressed on the grams per region basis the rubber content invariably either remained constant or showed increases from one sampling date to the next so that no losses in rubber content are indicated.

The data show that growth and rubber accumulation in guayule tissues are not necessarily antagonistic since rubber increases were observed even during the period when plants resumed growth. It should be emphasized that the statement of LLOYD (**12**) that the rate of rubber secretion varies inversely with the rate of growth has been substantiated by the work of KELLEY, HUNTER and HOBBS (**10**) and others. The present data do not contradict any such principle for it concerns rate of rubber accumulation.

TABLE VI

EFFECT OF DEFOLIATION ON MEAN PERCENTAGE RESINS, OF PLANT ORGANS AS INDICATED; GUAYULE PLANTS GROWN IN GREENHOUSE, SALINAS, CALIFORNIA, 1944–1945

PLANT ORGAN	SAMPLING DATES	PERCENTAGE DRY WEIGHT†			DIFFERENCE REQUIRED FOR SIGNIFICANCE			
		NOT DEFOLIATED	ONCE DEFOLIATED	REPEATEDLY DEFOLIATED	MEANS*		INTERACTIONS*	
					0.05	0.01	0.05	0.01
		%	%	%	%	%	%	%
Original leaves and new top growth‡	Oct. 21	5.39	5.32	5.35	0.38	0.51	.....	.....
	Dec. 19	4.83 (−0.66)§	4.21	4.66				
	Jan. 19	4.74 (−0.65)	4.44	4.82				
Original stem and branches	Oct. 21	8.60	8.63	8.40	0.53	0.71	0.75	1.01
	Dec. 19	5.63	5.09	6.02				
	Jan. 19	6.01	5.95	5.74				
Original 6″ roots	Oct. 21	8.69	9.01	9.11	0.48	0.64	0.67	0.90
	Dec. 19	6.82	6.04	6.18				
	Jan. 19	5.67	5.20	5.41				
New roots	Dec. 19	1.70	1.55	1.49	0.30	0.41	.....	.....
	Jan. 19	3.07	3.28	2.20				

* If values for treatments differ by more than the figure given in these columns, the odds are greater than 19:1 (0.05) or 99:1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten randomized 15-plant plots.

‡ Original leaves at beginning of experiments, and total original leaves plus new growth, stem and leaves in case of plants not defoliated and total new growth, stem and leaves, in case of defoliated plants, up to the dates indicated.

§ Values in parentheses indicate gain or loss over original dry weights of leaves.

Changes in resins content

The changes in resins content for the various tissue fractions are summarized in tables VI and VII. In the former, the data are expressed in percentage of total dry weight, and in the latter in grams of resins present in the regions of new growth, original stems, original 6-inch roots, and new roots.

The resins content of new growth, on the percentage basis, from Dec. 19 to Jan. 19, shows no significant change for treatments I and III, but a significant increase is indicated for treatment II. However, on the basis of grains

TABLE VII

Effect of defoliation on mean amount of resins, in grams per plot, of plant organs as indicated; guayule plants grown in greenhouse, Salinas, California, 1944–1945

Plant organ	Sampling dates	Grams per plot†			Difference required for significance			
		Not defoliated	Once defoliated	Repeatedly defoliated	Means*		Interactions*	
					0.05	0.01	0.05	0.01
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Original leaves and new top growth‡	Oct. 21	0.85	0.83	0.82	0.083	0.111	0.117	0.157
	Dec. 19	0.90 (+0.05)§	0.57	0.30				
	Jan. 19	1.54 (+0.69)	1.34	0.46				
Original stem and branches	Oct. 21	4.07	3.95	3.95	0.27	0.36	0.38	0.51
	Dec. 19	2.54	2.03	2.31				
	Jan. 19	2.42	2.43	2.06				
Original 6″ roots	Oct. 21	2.13	2.20	2.14	0.17	0.22	.....	.....
	Dec. 19	1.62	1.46	1.34				
	Jan. 19	1.40	1.28	1.09				
New roots	Dec. 19	0.01	0.03	0.01	0.004	0.005	.....	.....
	Jan. 19	0.15	0.27	0.03	0.029	0.039	.....	.....

* If values for treatments differ by more than the figure given in these columns, the odds are greater than 19:1 (0.05) or 99:1 (0.01) that the differences are not due to chance variation.

† Values reported represent means of ten randomized 15-plant plots.

‡ Original leaves at beginning of experiments, and total original leaves plus new growth, stem and leaves in case of plants not defoliated and total new growth, stem and leaves, in case of defoliated plants, up to the dates indicated.

§ Values in parentheses indicate gain or loss over original dry weights of leaves.

of resins per plot, a significant increase is shown for treatments I and II, but not for treatment III.

In the case of original stems, from Oct. 21 to Dec. 19, there is a very significant decrease in resins content ($P = 0.01$) on the basis of percentage dry weight and grams per plot. However, from Dec. 19 to Jan. 19, such a decrease ($P = 0.01$) holds only for stems of treatment II.

There is a continued decrease in resins ($P = 0.01$), for 6-inch roots, during the entire experiment, from Oct. 21 to Jan. 19, on the percentage and grams per plot bases, except that for roots of treatments I and II on a gram per plot

basis from Dec. 19 to Jan. 19, a decrease can be shown only on the $P = 0.05$ level of significance.

New roots, from Dec. 21 to Jan. 19, for treatments I and II showed increases in resins, but this was not the case in treatment III where plants were grown under a very low level of carbon assimilation.

Relation of rubber and resins changes

In figure 4, the changes in rubber and resins contents for the various regions of the plant, on the basis of grams per plot, are plotted against time.

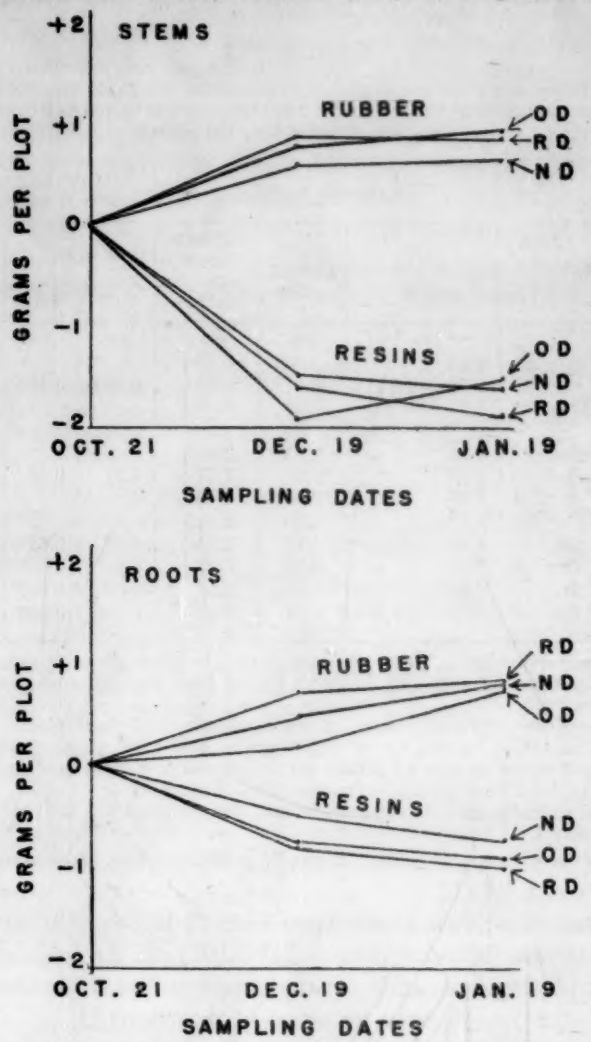

Fig. 4. Gain (+) or loss (−) in rubber or resins content of guayule stems and roots, on grams per plot basis; treatments I (ND) not defoliated, II (OD) once defoliated, and III (RD) repeatedly defoliated.

This shows unmistakably a negative correlation between changes in these two fractions in guayule tissue during the period when growth is resumed. It has already been pointed out (fig. 3) that the loss in dry weight of roots for treatment III, approximately equals that of the total carbohydrate fractions determined, but that in the stems, for treatment III, the loss in dry weight is greater than the latter total. Since the negative correlation shown in figure 4 is true for both roots and stems, it appears that during this period, the increase in rubber may be at the expense of some fraction or fractions of the resins (a mixture) functioning as rubber precursors. Other possibilities are considered in the succeeding section.

Discussion

The results presented show above all that the technique devised for growing guayule plants under a very low level of carbon assimilation (treatment III) in comparison with plants grown under a normal level of carbon assimilation (treatment II), was effective in starving the plants to such an extent that the more readily available water-soluble carbohydrates were practically depleted, and even part of the pentosans were used up. MURNEEK (**16**) has reviewed the subject of the rôle of hemicelluloses in woody plants, including his own contributions, which indicate that these complex carbohydrates "are not merely aggregates of the plant cell wall but also serve as reserve substances." SPOEHR (**23**) suggested that hemicelluloses are primarily pentosans. The present results as shown in figure 3, indicate that in the guayule stem, in addition to the pentosans which are only partially used as food even under conditions of severe starvation, in treatment III, there are still other substances present that may serve as food reserves and which are more readily available than the pentosans. What these other substances are is not known but they might consist of pectins.

The data also show, that in spite of the fact that the plants were under this extreme food (carbon) deficit, the rubber content, on the basis of grams per plot, never decreased but rather increased during the course of the experiment. The function of rubber in guayule tissues, whatever it may be, does not appear to be that of a food reserve.

It may be that rubber performs some other function in the economy of the plant that has aided it in its struggle for survival. The guayule plant is one of the most efficient rubber factories ever devised by nature for, from the wild, strains have been selected that produce more than 18 per cent. of pure rubber hydrocarbon (caoutchouc) on the plant dry weight basis in a few years under cultivation. If rubber were merely an inert constituent serving no function in the economy of the plant, such a large amount of excess baggage in the cells might be a distinct handicap in the competitive struggle for existence. On the basis of the present data, however, we can only conclude that rubber apparently does not function as a reserve food in guayule. What other function, if any, it may perform in the plant is still an open question.

The fact that increases in rubber, under all treatments, were associated with decreases in resins content in the tissues of the plant, suggests the possibility that a constituent of the resins fraction might serve as a rubber precursor. It should be emphasized, however, that the resins fraction, a mixture, would include also any such compounds as essential oils and fats that might be present. Unfortunately, only a few of the constituents of the resins fraction have as yet been identified as shown by the summary of TRAUB (**24**), and, therefore, it is not possible to point to a compound, or compounds, that might be considered from the standpoint of rubber precursors. According to PROKOFIEFF (**17**) and HAAGEN-SMIT (**8**) such a precursor would more likely be an aliphatic than a cyclic compound.

Summary

An experiment was conducted to determine the effect of carbon assimilation levels, ranging from very low to the normal, on the utilization of reserve foods in the guayule plant.

Guayule plants grown under a very low level of carbon assimilation by repeated removal of new top growth, (a) depleted their reserves of free sugars, levulins and inulin almost to 0; (b) utilized part of the pentosans; (c) lost no rubber hydrocarbon (caoutchouc), but showed a significant increase which was associated with a corresponding decrease in the resins fraction (a mixture), and (d) in the stems, lost a greater amount in dry weight than could be accounted for on the basis of loss in the water-soluble carbohydrates and pentosans determined.

The data show that: (a) the rubber hydrocarbon (caoutchouc) in the guayule plant apparently does not function as a food reserve, (b) the resins fraction (a mixture) may contain a rubber precursor, and (c) other reserves, such as pectins, might possibly be important food reserves and therefore deserve greater attention in physiological research.

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THE EXTENSION OF PLANT ROOTS INTO DRY SOIL[1]

ALBERT S. HUNTER AND OMER J. KELLEY

(WITH TWO FIGURES)

In the course of extensive fertilizer and irrigation experiments with guayule, there arose the questions of the ability of plant roots to extend themselves into dry soil and to absorb nutrients from such soil. These questions are of fundamental interest and of considerable practical importance, particularly in the case of the latter. The greatest concentration of available nutrient elements is usually in the topsoil, which is the first soil to become dry. If plants are not able to use water that is available to some other part of the root system for the absorption of nutrients by roots in "dry" soil, in some cases the elements added to the soil as fertilizers may be unavailable to the plant.

The literature records disagreement as to the ability of plant roots to extend into soil having moisture content below the permanent wilting percentage. HENDRICKSON and VEIHMEYER (**4**) concluded that the roots of sunflowers will not grow into soil which contains less moisture than is present at the permanent wilting percentage. SHANTZ (**8**) believed that the roots of some drought-resistant plants are able to penetrate dry soil, but that ordinary crop plants lack that ability. MAGISTAD and BREZEALE (**6**) and BREZEALE (**2**) made extensive studies of the moisture equilibrium between soil and plant and concluded that not only will roots elongate into dry soil, but the plant can absorb water through roots in moist soil, transport the water, and build up the moisture content of a dry soil to the wilting percentage.

An experiment was carried out in 1945 to investigate the penetration of roots into dry soil in atmospheres of varying degrees of relative humidity, and to study the uptake of nutrient elements from dry soil by roots of which a part were in moist soil. Corn was used as the experimental plant, in the belief that the findings would apply to other plants.

Experimental procedure and conditions

The photograph and schematic diagram of figure 1 show the arrangement of the component parts of the experimental apparatus. Sixteen pots of approximately 4-inch diameter and 8-inch height were prepared by brushing a mixture of equal parts of tar[2] (road oil) and paraffin (Parowax) on coarse cheese cloth, which was then shaped into cylinders with crimped-on bottoms. Examples of these pots are shown in figures 1 and 2. The walls and bottoms

[1] Special Guayule Research Project, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Salinas, California.

[2] The use of road oil as a component of this mixture was suggested by DR. A. C. HILDRETH.

were approximately $\frac{3}{16}$-inch thick. Before use the pots were filled with water and tested several days for leaks. All were water-tight. In order to further test the impermeability of the tar-paraffin mixture to water three air-dry Bouyoucos blocks (1) were coated with approximately $\frac{1}{4}$-inch of the material and immersed in water at room temperature for two weeks. Throughout the test the resistances of the blocks were above 250,000 ohms, indicating that no water penetrated the coating.

The pots were filled with a moist greenhouse potting soil and surrounded with approximately $1\frac{1}{2}$ inches of packed "dry" soil contained in wire mesh baskets lined with commodity cloth. One of the wire baskets with pot

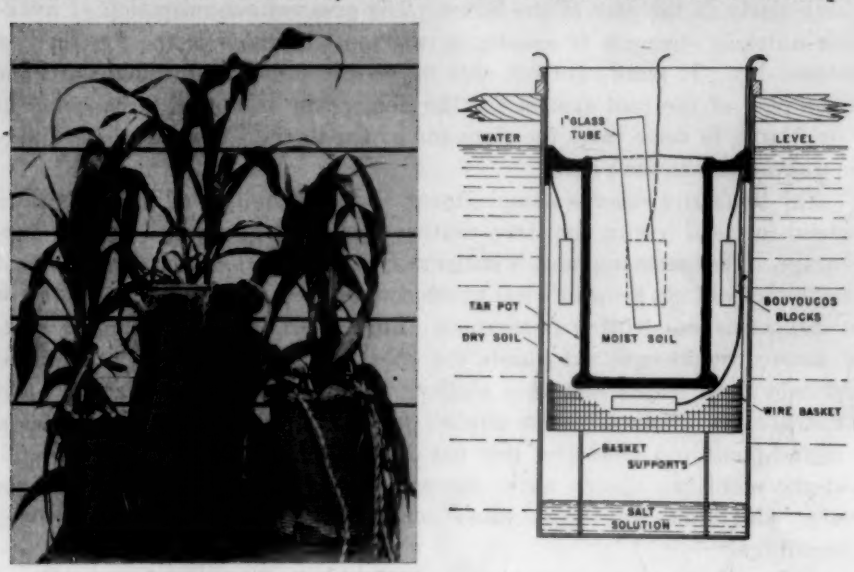

FIG. 1. Photograph and schematic diagram showing arrangement of component parts of experimental apparatus.

installed is shown in figure 1 (left). Approximately 4 kg. of air-dry Chualar loam, containing 0.4 per cent. moisture initially, was used with each pot. Its permanent wilting percentage and "field capacity" were approximately 4 and 12 per cent., respectively. With this soil was mixed approximately 0.5 kg. of dry quartz sand upon which a solution of radiophosphorus[3] had been dried. Four Bouyoucos blocks were spaced approximately equidistantly, in the air-dry soil, around the pot circumference, about midway between top and bottom and approximately $\frac{3}{8}$ inch from the pot wall. A similar block was placed centrally beneath the pot and another was installed inside it.

The screen baskets were supported 4 inches from the bottom of steel

[3] The radiophosphorus (as sodium phosphate) was supplied to us through the kindness of DR. JOSEPH G. HAMILTON, Crocker Radiation Laboratory, University of California, Berkeley, California. The initial and final (after 30 days) quantities of radiophosphorus were approximately 17 and 3 microcuries per pot, respectively.

cans 18 inches tall. There was approximately ¼ inch of free air space between the walls of can and basket. The rims of the pots were sealed to the walls of the cans by means of collars of cloth to which the tar-paraffin mixture was liberally applied. These seals confined the "dry" soil to the atmosphere in the space between the tar pot and the steel can. Four different conditions of relative humidity, about the "dry" soil surrounding the tar pots, were obtained through the addition of salt solutions to the bottoms of the steel cans, below the wire baskets. Each condition was replicated four times. These conditions are listed in table I.

To avoid fluctuations in relative humidity due to changes in temperature, the 18-inch steel cans were immersed to a depth of 15 inches in a constant-temperature tank which has been described by CAMPBELL and PRESLEY (3). Throughout the test period the temperature was maintained at 84 ± 2° F. (except for one day, when a maximum of 88° F. was reached).

TABLE I

CONDITIONS OF RELATIVE HUMIDITY ABOUT THE "DRY" SOIL SURROUNDING THE TAR POTS

SERIES	POTS	ESTIMATED MAXIMUM RELATIVE HUMIDITY	ESTIMATED MINIMUM MOISTURE TENSION†	OBTAINED BY ADDING TO CAN
		PERCENTAGE	ATMOSPHERES	
A	3, 6, 12, 13	50	950	No solution
B	2, 5, 11, 16	82*	275	1 liter saturated NaCl (approx. 6 M)
C	1, 8, 10, 15	99*	15	1 liter 2.2 M NaCl
D	4, 7, 9, 14	100	0	1 liter water

* Calculated from Raoult's Law, $p = p_o N_1$.

† Calculated from the formula, tension in atmospheres $= \frac{RT}{0.018} \ln \frac{100}{\text{relative humidity}}$.

Three corn plants were grown in each pot. Water was added as required to the soil inside the pot to maintain a low moisture tension (resistance of Bouyoucos blocks around 500 ohms, or less). The resistances of the Bouyoucos blocks in the "dry" soil were determined at frequent intervals, usually two days, throughout the 30-day duration of the test.

The actual atmospheric conditions in the "dry" soil of the four series of pots were not determined. RUSSELL (6) indicates that the relative humidity of air-dry soil is about 50 per cent. The values of 82, 99, and 100 per cent. presented in table I are the highest values theoretically possible in equilibrium with the given solutions; it is very doubtful that values as these were actually reached during the test period. The estimated moisture tensions are the minimum values attainable in soil in equilibrium with the given maximum relative humidities.

Results and discussion

Twelve days after germination of the corn, pots 14 and 15 were removed for examination. Several roots had penetrated the pot walls and extended

into the "dry" soil for about half an inch. The average moisture content of two separate samples of soil from the immediate vicinity of the roots was 1.04 per cent. for pot 14 and 0.80 per cent. for pot 15, as compared with an initial content of 0.4 per cent. The resistance of the Bouyoucos blocks was of the order of 35,000 ohms for pot 14 and 125,000 ohms for pot 15.

Pot 1 was examined after 24 days. Numerous roots had grown into the "dry" soil for one to two inches. The average moisture content of the "dry" soil about the roots was 1.12 per cent. and the average resistance of the Bouyoucos blocks was 31,000 ohms.

FIG. 2. Photographs showing corn roots that penetrated the walls of the tar pots and extended into "dry" soil at varying degrees of possible relative humidity, as follows: Upper left, Series A, approximately 50 per cent.; lower left, Series B, 82 per cent.; upper right, Series C, 99 per cent.; lower right, Series D, 100 per cent. Thirty days after germination.

After 30 days all the remaining 13 pots were removed and photographed. In every case, regardless of the conditions of relative humidity, many roots had extended into the "dry" soil, as is shown in figure 2. The surfaces of the roots were moist with exudate and in most cases had a thin sheath of adhering soil grains. Such a sheath was frequently observed by MAGISTAD and BREZEALE (**6**). That the soil about the roots was "dry" is evidenced by the fact that no brushing or other treatment was needed to free the roots of soil as they are shown in the photographs. Throughout the experimental period the resistances of the Bouyoucos blocks in the "dry" soil about these 13 pots were of the order of 250,000 ohms. BOUYOUCOS and MICK (**1**) state that the resistance at the permanent wilting percentage is around 100,000

ohms. Two samples (of 200–300 gm. each) of "dry" soil were taken from about each pot for moisture determination. The maximum, minimum, and mean percentage values, respectively, were as follows: Series A, 1.33, 0.70, and 1.00; Series B, 1.71, 1.11, and 1.29; Series C, 1.58, 1.16, and 1.27; and Series D, 1.25, 0.36, and 0.86.

All these moisture values are far below the permanent wilting percentage and all indicate a build-up of moisture in the "dry" soil. Since no water or solution was present in the cans of Series A, and the build-up of moisture in the soil of that series was practically as great as in either of the other series, the build-up in the "dry" soil must be attributed to the presence of roots rather than to absorption of water by the soil from the atmosphere surrounding it.

The tar-paraffin mixture was soft and plastic at the temperature of the experiment. The roots were continually growing and expanding, which tended to maintain extremely intimate contact between the root surface and the periphery of the hole in the pot wall at the point of penetration, preventing the seepage of water, alongside the roots, through the pot wall. Moreover, the surface forces of the "dry" soil, and its attraction for water, as the roots entered it, were much greater than the surface forces of the plant root. In such a case a film of water would not flow along a plant root surface through the "dry" soil, but would be "pulled" from the root surface and absorbed by the first dry soil with which it came in contact. For similar reasons water poured on a column of dry soil does not flow freely downward through any root channels or other openings that may exist, but is absorbed by the dry soil and moves downward as a moisture front. There is no reason to think that the build-up of moisture in the "dry" soil of this experiment was due to the flow of water along the root surface from the moist soil inside to the "dry" soil outside the pots. It must be attributed to the conductance of water through the tissues of the roots.

From the standpoint of the moisture stresses involved it is to be expected that plant roots will give up water and build up the moisture content of soil in which the moisture tension (or negative pressure) is higher than that of the root sap. The flow of water in plant roots is not polar. It is a familiar fact that water moves out of plant tissues and plasmolysis occurs if they are placed in solutions having greater osmotic pressures than the cell sap. MAGISTAD and BREZEALE (**6**) believed that there is an equilibrium between the plant and the soil, and with some plants at least, water moves either from the soil to the plant or from the plant to the soil, depending upon the nature of the moisture gradient. RUSSELL (**7**) states that the osmotic pressure of the root sap of most plants is of the order of 7 to 165 atmospheres as the soil moisture nears the wilting coefficient; for many plants it is around 15 to 20 atmospheres. The moisture tension in air-dry soil is around 1000 atmospheres (**7**); in soil at the permanent wilting percentage it is approximately 15 atmospheres. The fact that the soil moisture tension and the osmotic pressure of the root sap are both approximately 15

atmospheres in soil at the permanent wilting percentage may explain why plant root systems can build up the moisture content of dry soil to the wilting percentage but not above it, as BREZEALE (2) observed.

At the end of the experiment the aerial portion of the corn plants was tested with a Geiger counter for the presence of radiophosphorus. None was detected. This, however, cannot be taken as proof of the inability of roots to absorb nutrients from dry soil when moisture is available to them elsewhere. BREZEALE (2) showed that wheat seedlings were able to absorb potassium from soil initially at the permanent wilting percentage when water was available elsewhere in the root system. Had the corn been permitted to grow for a longer period, absorption of phosphate from the "dry" soil might have occurred. Relatively little of the "dry" soil was in contact with the roots. There is no way of estimating with any accuracy the amount of radiophosphorus in sufficiently close contact with the roots for absorption to take place under favorable circumstances. The presence of a probably ample supply of phosphorus in the moist greenhouse potting soil mixture in which the plants grow is a further factor which may have inhibited absorption of radiophosphorus from the dry soil. The results of this test are not conclusive as regards nutrient absorption from initially dry soil, but it is felt that a technique similar to the one employed can be used to answer the question. The authors attempted by another technique (5) to study the absorption of nutrients from soil in the wilting range.

Summary and conclusions

The extension of plant roots into dry soil was investigated. Corn was grown for 30 days in tar-paraffin pots filled with moist soil and surrounded by air-dry soil containing radiophosphorus. Bouyoucos blocks were installed in the "dry" soil for the periodic determination of its moisture condition. Variable conditions of relative humidity were maintained about the "dry" soil.

In all cases the corn roots penetrated the walls of the pot and extended into the "dry" soil. The moisture content of the "dry" soil increased, but values as high as the permanent wilting percentage were not obtained.

The results of the experiment indicate that the roots of corn are able to elongate into dry soil and to build up the moisture content of that soil. No evidence was obtained for the absorption of nutrients from dry soil by plants.

SPECIAL GUAYULE RESEARCH PROJECT
U. S. DEPARTMENT OF AGRICULTURE
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THE EFFECTS OF LIGHT, GRAVITY, AND CENTRIFUGAL FORCE UPON THE TROPIC RESPONSES OF BUCKWHEAT SEEDLINGS[1]

H. L. Chance and James M. Smith

Introduction

The tropistic responses of plants have long interested botanists. They are so evident that one of the earliest botanists observed they could "scarcely have escaped the notice of the most inattentive observer of vegetation."

Went and Thimann (3) state that as early as 1758 Du Hamel proved the radicle of an inverted seed would reverse and turn downward while the plumule turned upward. He also conceived the idea of two saps, one moving upward and the other downward, controlling the growth. Knight (2) decided that if, as some naturalists thought, the directional growth of plant shoots is caused by gravity, its effect might be suspended by the action of centrifugal force. In his well-known experiments with seeds of the common garden bean fastened on the perimeter of a wheel and rotated, he found that the shoots grew toward the center of the wheel. They even grew past the center, but then reversed, growing toward the center of the wheel again. He also found that the angle of the shoots from the perpendicular increased with an increase in the speed of the rotation of the wheel.

These experiments were made in an attempt to correlate quantitatively the influence of gravity, centrifugal force, and light upon the directional growth of shoots. Gravity is a fixed factor for a definite earth position and is used as a standard of comparison. The centrifugal force was developed by a wheel having a radius of 35 centimeters. The light source was a 100-watt Mazda lamp in 110–115 volt circuit. Since stimuli act through time, the unit taken as a standard was the time required for shoots placed in a horizontal position in the dark to assume a vertical position; *i.e.*, three hours. The temperature for all experiments ranged from 26° to 28° C.

The giant form of the Japanese variety of buckwheat (*Fagopyrum esculentum*, Moench) was chosen since preliminary experiments indicated giant forms to be more sensitive than dwarf forms. The seeds were selected and placed in moist chambers to germinate and seedlings were selected from these.

The plants were grown in soil consisting of two-thirds sand and one-third loam. The soil was placed in ten-ounce paper cups and the germinated seeds planted in a row across each cup. Two 200-watt Mazda lamps were placed eighteen inches above the cups to facilitate straightening of the seedlings as quickly as possible after emerging from the soil. The plants were usually grown and prepared in the same room in which the tropic reactions were studied. When ready for use, the shoots were thinned to

[1] Contribution from the Department of Plant Sciences, University of Oklahoma, no. 84.

three or four in each cup by clipping off near the soil. The remaining shoots were selected for straightness, size, and spacing. Their length was then determined and the shoots were ready for use. After being subjected to treatment for three hours, their curvatures were measured by means of a protractor such as used and illustrated by WENT and THIMANN (**3**).

In experiments using centrifugal force a plywood platform, thirty-six inches in diameter, was constructed on a steel wheel, formerly part of a wheel chair. A circle, concentric from the center of the wheel, with a radius of 35 centimeters was drawn on the platform. With a known radius the centrifugal force could be computed in terms of gravity according to the formula:

$$\text{C. F.} = 0.0000112\ W^2r$$

When C. F. = Centrifugal Force
W = Revolutions per minute
And r = radius in centimeters

Eighteen metal cups, containers for the culture cups, were spaced around and outside the circle in such a position as to allow the top of the culture cups to be flush with the line. The metal cups were fastened on the platform with "L"-shaped iron strips. They were secured to the strips by a bolt passing through a slot about an inch long cut in the strips and cups. This device made it possible to adjust the metal cups to any desired angle. In order to prevent air currents and light from passing from one side across center to the other side, nine partitions of black window shade cloth supported by wire frames were placed on the platform radiating from the center to perimeter, thus dividing the platform into nine equal wedge-shaped compartments. There were two metal cups in each compartment. A sun-ray glass (trade name for ordinary screen wire covered with translucent plastic material) screen encircled the wheel to the height of the partitions. A lid of cardboard covered with black cloth was used in experiments in the dark. The wheel, turned by a one-third-horsepower motor, was not operated directly from the motor, but from an intervening set of pulleys of various sizes depending upon the speed desired.

Methods and results

The response time was arrived at by placing the plants in a dark room with the shoots in a horizontal position and noting the time required for the shoots to assume a vertical position. The basement lights were turned on and the measurements made immediately. The time required was three hours. This was used as the reaction time for all other experiments.

It was next desirable to determine the least centrifugal force required to influence directional growth. If plants are placed in a vertical position on a wheel revolving in a horizontal plane, gravity influences curvature. To overcome this influence the wheel was revolved in a vertical plane with shoots at right angles to the radius of the wheel. The wheel then served as

a klinostat and gravity was neutralized. The speed required for the wheel to act as a klinostat was not determined but only the speed necessary to produce a centrifugal force sufficient to influence curvature. The speed was found to be between 7 and 8 r.p.m. or 0.019 to 0.025 earth pulls. There was no response at 0.019 earth pulls and at 0.025 earth pulls 33 of the 161 shoots gave no curvatures, while the remainder gave definite responses. The curvatures for the individual shoots varied from 0° to 13° with an average of 5.46°. This figure is based on four separate experiments. The centrifugal force equivalent to 0.025 of an earth pull was taken as the sensitivity point for these shoots. The results compare favorably with those of DARWIN and PERTZ (1) who found that the plants with which they worked responded to a centrifugal force of 0.02 to 0.05 earth pulls.

Though KNIGHT (2) found that the angle of shoots from the perpendicular increased with the speed of the wheel, he made no attempt to measure curvature at known speeds. To make these determinations the wheel was changed to a horizontal position for all other work. The wheel was adjusted while loaded with cups of soil, thus giving it the same load it would have during the experiments, to the speed at which the experiment was to be made. The cups containing the plants were numbered; each shoot was measured and its length recorded when placed on the wheel. At the end of the response time, the angle of curvature was recorded for each shoot.

A third factor to determine was the location of the point of balance between gravity and centrifugal force in the dark. Substituting in the formula C. F. = 0.0000112 W^2r, it was found that the wheel must rotate 50.5 + r.p.m. to develop a centrifugal force equivalent to an earth pull. Theoretically, plants placed at any angle and rotated at this speed should give angles which would bring them to a line parallel to the resultant of the two forces or to an angle of 45°.

One hundred fifty-seven plants, representing four separate experiments, were placed on the wheel in a horizontal position and rotated at 50.5 + r.p.m. for three hours. The average curvature was found to be 44.93° upward (table I).

This was repeated with the plants placed in a vertical position. The shoots gave an average curvature of 46.06° downward or 43.94° from the horizontal. When 66 plants were placed on the wheel at an angle of 65° from the vertical, the average curvature was found to be 21.01° upward. This angle, plus the angle of the shoots at the beginning, 25° from the horizontal, gives a total of 46.01° from the horizontal. Fifty-six plants were placed at an angle of 35° from the vertical and rotated as above. The average curvature was 12.05° downward. The angle at the beginning, 35° from the vertical, plus the angle of curvature, makes a total of 47.05° downward or 42.95° from the horizontal. Thus for the four positions, *i.e.*, horizontal, vertical, 65° from the vertical, and 35° from the vertical, the final positions taken by the shoots after treatment were 44.93, 43.94, 46.01 and 42.95° upward from the horizontal positions or a variation of slightly more than three degrees.

The curvatures were also determined for both horizontal and vertical positions of shoots subjected to 0.96 earth pulls which were obtained at a speed of 49.5 r.p.m. One hundred ninety-eight plants placed in a horizontal position and rotated for the reaction time gave an average curvature of 50.70° upward. One hundred fifty-eight plants placed in a vertical position were rotated as above and gave an average curvature of 37.39° downward. The response in both cases was in accordance with the assumption that the shoots would give curvatures in the direction of the greater stimulus. This point was further established when the shoots were subjected to a centrifugal force of 1.04 earth pulls. One hundred and one shoots placed in a horizontal position gave an average curvature of 40.46° upward while 157 shoots placed vertically gave an average curvature of 50.59° downward.

TABLE I

SUMMARY OF ALL CURVATURES FROM THE HORIZONTAL POSITION OF PLANT SHOOTS POINTING TOWARD THE CENTER OF THE WHEEL WHEN ROTATED AT 50.5+ R.P.M. (1 EARTH PULL). TEMPERATURE 26–28° C.

LENGTH OF SHOOTS	NO. OF PLANTS	38°	39°	40°	41°	42°	43°	44°	45°	46°	47°	48°	49°	50°	51°	52°
cm.																
2½	14	....	1	1	....	1	....	3	3	1	1	1	2	....	....	....
3	25	1	....	....	2	....	2	3	7	5	2	....	1	1	....	....
3½	34	....	1	2	2	1	4	3	4	4	6	3	1	2	1	....
4	36	....	2	1	....	3	1	5	9	4	2	1	5	2	1	....
4½	19	....	1	2	....	....	2	....	6	7	....	....	....	....	....	1
5	16	....	....	....	3	1	2	1	2	....	2	2	2	....	1	....
5½	10	....	....	....	1	....	....	2	1	2	....	....	2	....	2	....
6	3	....	....	....	....	....	2	....	1	....	....	....	....	....	....	....
Totals	157	1	5	6	8	6	13	17	33	23	13	7	13	5	5	1

Average curvature 44.93 degrees upward.

The preceding experiments were carried out in the absence of light. Next it was desired to add this factor to similar experiments. In order to do so, it was necessary to determine the influence of light as compared to that of gravity. This was done in two ways. First the plants were placed upright on the floor and illuminated unilaterally at various distances from a 100-watt Mazda lamp which was placed in a black box with an open end toward the plants. Cups containing the plants were placed at various distances from the light source and left for the three-hour reaction period and the curvatures determined. The distance from the light source at which the shoots (30 in number) approached an average angle of 45° in the reaction time was 120 centimeters. The individual shoots at this distance varied in curvature from 43° to 48° with an average of 44.6°. In the second method the plants were placed in a horizontal position at various distances above the light source. Again the point of balance was found to be 120 cm. from the light source. At this distance the shoots of six plants gave no curvature while those of twelve plants curved upward and thirteen downward.

In balancing light plus gravity against centrifugal force, it was necessary to use two sets of experiments in order to keep the light source the same distance from the plants. The first experiments were run with the plants in a horizontal position with the light source 120 cm. above the plants. In the second group of experiments the plants were placed upright which necessitated moving the lights upward a corresponding distance.

Four lights instead of one were used, the lamps being equally spaced around the wheel above the plants. A shade was arranged around the lights to restrict the illumination and prevent reflections which would strike the plants from the sides. Cross partitions were used to separate the four lights to prevent the rays from crossing and giving more illumination than was afforded by one light.

When the plants were placed in a horizontal position and the light source as described placed 120 cm. above, the shoots were subjected to one earth pull plus its equivalent in light. Theoretically a centrifugal force of two earth pulls would be required to balance the two forces and was obtained by rotating the wheel at a speed of approximately 72 r.p.m. Four experiments were run at this speed with the plants in a horizontal position. The average curvature of the 229 shoots involved was 45.25° upward. Six experiments were made with the plants in a vertical position. The average curvature for a total of 356 shoots was 45.71° downward. The results substantiate the hypothesis that the stimuli of gravity and light are directly additive in respect to centrifugal force in effect upon the curvatures. This principle was further borne out by checking the curvatures of shoots at approximately 1.9 and 2.1 earth pulls in terms of centrifugal force. Four experiments were made consisting of 276 plants placed in a horizontal position and subjected to a centrifugal force of approximately 1.9 earth pulls. The average curvature of the shoots was 50.94° upward; of the two hundred eighty-nine plants placed vertically and treated as above, the average curvature of the shoots was 41.16° downward. Next 300 plants were placed in a horizontal position and subjected to a centrifugal force equivalent to approximately 2.1 earth pulls. The average curvature of the shoots was 38.39° upward, while 292 plants placed in a vertical position and given similar treatment gave an average curvature of 52.70° downward.

It was found that the tropic influence of gravity plus its equivalent in light could be counteracted by a centrifugal force of two earth pulls. Under this condition the shoots of those plants placed in a horizontal position averaged an angle of 0.25° above while those in a vertical position averaged an angle of 0.71° below that of the balance point. As could be expected, the shoots of the plants subjected to a centrifugal force of 1.9 earth pulls did not come to a position of 45°, but to an angle above 45°, thus giving the greater response to the greater stimuli of gravity and light. The shoots of the plants in a horizontal position averaged an angle 5.94° above the point of balance while for those in a vertical position it was 3.84° above the balance point. When the plants were subjected to a centrifugal force of 2.1 earth

pulls, the shoots again gave a greater response to the greater stimulus. The shoots placed in a vertical position were brought to position of 7.70° below the balance point while those in the horizontal position were brought to positions averaging 6.61° below the balance point of 45°.

The reaction time of shoots was determined in respect to gravity plus its equivalent in light. The plants were placed in a horizontal position and the light source placed 120 cm. above. The time required for the shoots to change from a horizontal to a vertical position was one hour and fifty minutes whereas it required 3 hours in the absence of light. These data do not indicate that gravity and light are directly additive in respect to time in their combined influence upon curvatives.

The next step was to balance light against centrifugal force but due to mechanical difficulties this experiment was abandoned. However, since gravity and its equivalent in centrifugal force have been found to have comparable effects and gravity plus its equivalent in light have been found to be additive as to centrifugal force, then light should balance centrifugal force in its tropic effect upon buckwheat seedlings. This leads to the assumption that light, gravity, and centrifugal force can be expressed in the same terms of earth pulls in considering their effects on tropic responses of buckwheat shoots.

Summary

1. The time required for the shoots of buckwheat seedlings placed in a horizontal position, in the dark and at a temperature of 26–28° centigrade, to assume a vertical position was three hours.

2. The least centrifugal force exerted in a vertical plane required to induce tropic response in buckwheat shoots was found to be between 0.019 and 0.025 earth pulls which required about 8 r.p.m. when shoots were 35 cm. from the axis of the wheel. The average curvature at this speed was 7.16 degrees. At 0.019 earth pulls, the shoots gave no response.

3. When a centrifugal force equivalent to one earth pull was exerted upon shoots in a horizontal position the shoots assumed a position parallel to the resultant of the two forces or an angle of 44.93°, as compared to the theoretical of 45°. The shoots of the plants placed in a vertical position and subjected to the same treatment gave an average curvature of 46.06° downward. When the centrifugal force was changed to 0.96 earth pull, the shoots in the horizontal position gave an average curvature of 50.70° upward while those placed in a vertical position gave an average curvature of 37.39° downward. If the centrifugal force was changed to 1.04 earth pulls, the shoots in a horizontal position assumed an average angle of 40.46° upward and those placed in a vertical position 50.59° downward.

4. The distance at which shoots approached an angle of 45° in the reaction time of three hours when unilaterally illuminated was 120 cm. from the light source, when using a 100-watt Mazda lamp in 110–115 volt circuit. The average of the angles at this distance was 44.6 degrees.

5. The distance from the light source at which the shoots gave the least

curvature in the reaction time when illuminated from below was 120 centimeters.

6. The influence of one earth pull plus its equivalent in light upon the curvature of shoots was offset or balanced by a centrifugal force of two earth pulls.

7. The reaction time of shoots placed in a horizontal position and illuminated from above with the light source 120 cm. away was one hour and fifty minutes. The difference in the reaction times of shoots in the dark and in the light was one hour and ten minutes.

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MICROMANIPULATIVE STUDIES ON GELATINIZED STARCH GRANULES

M. J. Cox and M. M. MacMasters

(WITH TWO FIGURES)

Gelatinized starch granules are generally considered to be comprised of sacs or outer membranes of starchy material enclosing a more or less fluid colloidal starch dispersion [see reviews by Radley (10)] and Kerr (6). Nägeli (9) and Meyer (8) considered the membrane to be precxistent in the native, ungelatinized granule. Alsberg (1) and Badenhuizen (2, 3) on the other hand, concluded that the membrane is formed as an artifact by the swelling and coalescence of the more resistant lamellae. Experimental results reported by Hess and Rabinowitsch (5) and further discussed by Roberts (11) indicate that, however it may be formed, the outer membrane of the gelatinized granule is tough and can be punctured by a needle, releasing a fluid colloidal suspension with which the sac is filled.

During the course of an investigation at this Laboratory of the factors affecting starch paste viscosity, micromanipulative studies were made of the degree to which gelatinized granules of corn, potato, tapioca, and glutinous corn starch could be stretched. Several hundred granules of each kind of starch were observed, each granule being stretched until broken. The main purpose of the study was to determine whether length of paste might be correlated with ability of the individual unbroken granules to stretch. (A long paste has a small yield value and low mobility.) Information was also obtained on the degree to which starch granules could be stretched after pasting at different temperatures and for different lengths of time. Some effects of chemical pretreatment of starch granules upon their subsequent ability to stretch were also noted.

The starches used in these experiments included commercial tapioca starch, laboratory-processed glutinous corn starch, and both commercial and laboratory-processed corn and potato starches. Nitrogen and ash contents of these starches, determined as described by MacMasters and Hilbert (7), are given in table I. The laboratory-processed samples of corn starches were prepared by the method described by Cox, MacMasters, and Hilbert (4).

Methods

In order to move and stretch the starch granules, fine glass needles were used in a pair of Fitz micromanipulators which permitted movement in three planes. Gelatinized starch granules were stretched at room temperature and at approximately 40° C. For stretching at room temperature a glass micromanipulator cell of conventional type was used. The gelatinized starch was suspended in a small drop of water on a cover slip and the slip

TABLE I

DESCRIPTION OF STARCH SAMPLES USED

SAMPLE NUMBER	STARCH	METHOD OF PREPARATION	PERCENTAGE ON DRY BASIS	
			N	ASH
			%	%
1	Tapioca	Commercial	0.01	0.09
2	Glutinous corn	Laboratory 0.1% SO_2 steep	0.04	0.04
3	Corn	Commercial SO_2 "	0.05	0.06
4	Corn	Laboratory H_2O "	0.03	0.07
5	Potato	Commercial No steep. SO_2 processed	0.01	0.03
6	Potato	Laboratory " " H_2O "	0.03	0.04

inverted over the opening in the center of the glass cell. A similar micro-manipulator cell heated by water circulated from a constant temperature bath was employed for samples stretched at 40° C.

Individual starch granules were stretched by placing two micro-needles upon the granule and slowly pulling the needles apart. The needles were placed near the center of the granule, then as they were pulled apart, they slipped over the surface of the granule until near the granule periphery. Thus stretch was measured throughout essentially the whole length of the granule. Considerable care was necessary in placing the needles upon a granule so as not to puncture it prior to stretching. When granules were biaxial, as in potato starch, they were pulled so that the stretch would be along the long axis. For the starches which had approximately spherical granules, alignment was necessarily at random. The needles were pulled apart slowly. Rapid or jerky movement of the needles caused the granules to break before the maximum degree of stretch was reached. When the granules were stretched and broken, the granule fragments would snap back around their respective needles. Measurements were made with a microscope equipped with a calibrated ocular micrometer. The percentage stretch of each granule was calculated.

$$\text{Percentage stretch} = \frac{\text{Stretched diam.} - \text{original diam.}}{\text{Original diam.}} \times 100.$$

Percentage of stretch within any one sample varied considerably. Twelve corn starch granules from a sample pasted for 10 minutes in a boiling water bath and stretched as soon as they had cooled to room temperature (25° C.) gave 55 to 222 per cent. stretch, average 127. Another 13 granules, prepared and stretched in an identical manner gave 100 to 207 per cent. stretch, average 150. For 140 corn starch granules, including the 25 already mentioned, the average stretch was 157 per cent. These granules were chosen at random from 11 different samples. These data were analyzed statistically and it was found that the probability is about 99 per cent. that the mean of the sample, 157, is within 10 of the mean of the population of starch granules; *i.e.*, it is practically certain that the mean of the stretch of starch

granules taken from similar starch pasted under similar conditions will fall within the range of 147–167 per cent.

Results and Discussion

Nature of the interior of starch granules

Roberts (**11**), in a résumé of work which he had carried out earlier with Hess (**5**), reported that with an ultramicroscope Brownian movement could be seen within gelatinized starch granules to which alcohol or other organic solvent had been added. Badenhuizen (**3**) reported that upon pricking an untreated swollen starch granule with a needle the granule did not crumple nor did any material flow out from it. Repetition of the work of these two authors confirmed the results of both. The apparent conflict

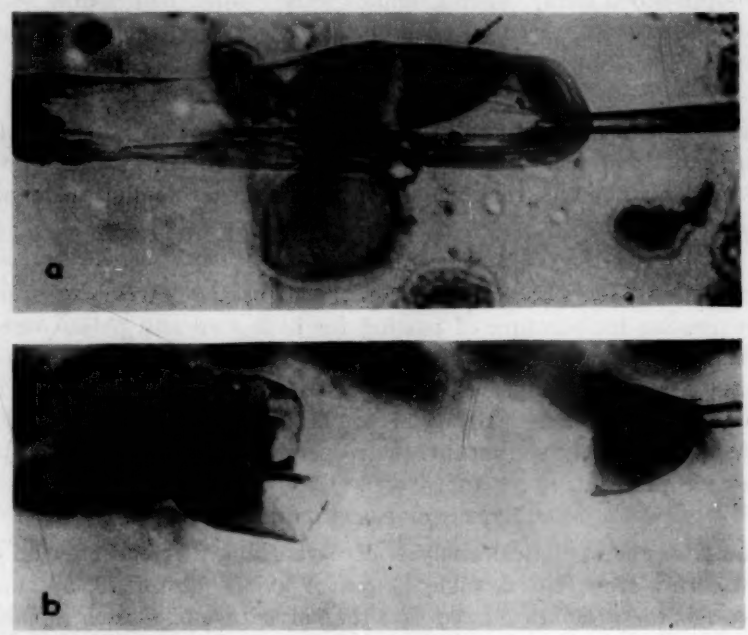

Fig. 1. Gelatinized potato starch granules stained with 1 per cent. I_2KI. a. Maximum amount of stretch. Note gelatinous center at tip of arrow. b. Granule broken and snapped back around needles. ×135.

in their results can be attributed to the use by Roberts of an organic solvent which causes precipitation of the colloidal material within the liquid contents of the granule.

Gelatinized potato starch stained with approximately 1 per cent. I_2KI solution appeared to have a firm gelatinous center (fig. 1a) around which there was a clear transparent margin. When these granules were stretched and broken, the break was clean (fig. 1b).

When 0.01 per cent. solution of I_2KI was used, the interior of the granule was not precipitated and the break was ragged. It was reported by Badenhuizen (**3**) that the iodine staining hardened the granules and made them

much more difficult to cut than unstained granules. The clear margin which surrounds the dark-stained center of the granule is attributed by BADENHUIZEN (3) to the thinness of the granule in this region.

In order to determine whether reorientation of the carbohydrate molecules within the starch granules could be brought about by stretching swollen starch granules, a commercial corn starch (No. 3, table I) was gelatinized in a boiling water bath and stretched between crossed Nicol prisms. The granules were stretched parallel to the axis of the polarizer and no birefringence was observed. The micromanipulator needles were then set approximately 30° from the axis, but still no birefringence was exhibited by the stretched granules. Although it is known that in many instances the addition of I_2KI solution will bring about a return of birefringence to gelatinized starch granules (unpublished data), when granules were stained with iodine solution and subsequently stretched between crossed Nicol prisms, no birefringence was seen.

Effect of temperature at time of stretching

The length to which granules could be stretched at 25° C. was not significantly different from that to which similar granules could be stretched at 40° C. This was true for each starch studied regardless of pasting temperature and time.

Effect of pasting temperature

A significant increase was found in the percentage stretch of granules with increasing temperature of pasting for both corn and potato starches. Corn starch granules pasted at 80° C. showed a mean stretch of 106 per cent. compared with 152 per cent. for those pasted in a boiling water bath. In the case of potato starch the difference between samples pasted at 80° C. and 90° C. was highly significant, the respective averages of stretch being 140 and 185 per cent.

Effect of pasting time

Results of the statistical analysis of data (table II) collected on commercial corn starch (No. 3, table I) pasted at 80° C. for 10, 30, and 60 minutes and for 72 hours showed that the percentage of stretch in samples

TABLE II

PER CENT. OF STRETCH OF STARCHES SHOWING NUMBER OF ITEMS IN SAMPLE AND THE STANDARD DEVIATION OF SAMPLES PASTED AT 80° C. FOR SPECIFIED LENGTHS OF TIME

STARCH/STATISTICAL MEASURE	TIME OF PASTING			
	10 min.	30 min.	60 min.	72 hours
Corn starch				
Mean stretch (%)	94	126	100	84
No. in sample	12	12	12	20
Standard dev.	23	34	27	45
Potato starch				
Mean stretch (%)	.....	135	.....	77
No. in sample	.....	50	.....	15
Standard dev.	.....	37	.....	18

pasted 30 minutes is significantly greater than that in samples pasted for all other lengths of time used (table III). No difference was shown among samples pasted for 10 minutes, 60 minutes, and 72 hours. From the available data, it is impossible to say at what time between 10 minutes and 1 hour maximum stretch would occur.

Although the data for potato starch (No. 5, table I) are incomplete, granules pasted for 30 minutes at 80° C. were found to stretch considerably more than granules from a similar sample pasted for 72 hours (table II). There was a highly significant difference in the length to which granules pasted for these two time intervals would stretch before breaking.

TABLE III

SIGNIFICANCE OF DIFFERENCE OF STRETCH BETWEEN SAMPLES OF CORN STARCH PASTED FOR VARYING LENGTHS OF TIME AT 80° C.

TIME OF PASTING	TIME OF PASTING			
	10 MINUTES	30 MINUTES	60 MINUTES	72 HOURS
10 minutes	—	*	†	†
30 minutes	....	—	*	‡ *
60 minutes	....	....	—	†
72 hours	....	....	....	—

* Indicates 5 per cent. level of significance.
† No significant difference.
‡ Approaches 1 per cent. level of significance.

RELATIONSHIP OF STRETCH TO STARCH PASTE VISCOSITY

One of the objects of this investigation was to determine whether there is any correlation between paste viscosity and the ability of individual granules to stretch. There is known to be a rough correlation between length of paste, tackiness, and paste viscosity. For example, corn starch which has a short paste has a low paste viscosity and tapioca and potato starch which have long tacky pastes have a high paste viscosity. In concentrated pastes, in which the granules might be in close contact with each other, the ability of individual granules to stretch might contribute materially to the length of the paste. From the data at hand, however, it would appear that there is no correlation between the ability of a starch granule to stretch and the paste viscosity of the starch.

The MacMichael viscosity (at 95° C., 20 r.p.m.) of 3 per cent. suspensions pasted at 95° C. for 10 minutes with stirring at 120 to 160 r.p.m. is 375, 270, 95, and 140 C.P. for glutinous corn, tapioca, corn and potato starches, respectively. Glutinous corn starch or tapicoa starch pasted for 10 minutes in a boiling water bath could be stretched to about 3 times or 200 per cent. of the original granule length (fig. 2) as compared with 157 per cent. for corn and 145 per cent. for potato starches.

The stretch of pasted granules of corn starch which had been processed with sulfur dioxide (No. 3, table I) was compared with the stretch of simi-

larly pasted granules of the same kind of starch processed with distilled water (No. 4, table I). The latter had higher viscosity. There was no more difference in stretch between these two starches than there was among granules within either sample. A portion of commercial corn starch (No. 3, table I) was shaken for one hour in N/32 NaOH solution and subsequently washed free of alkali. This treatment increased the paste viscosity of the

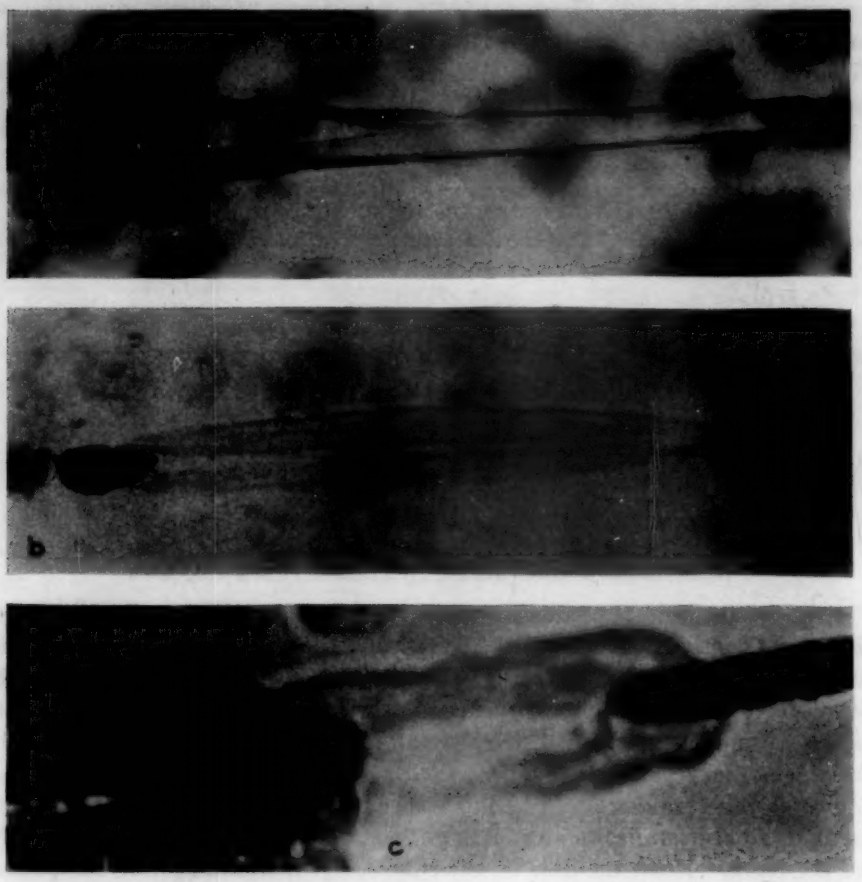

FIG. 2. Starch granules stained with 0.01 per cent. I_2KI showing maximum amount of streach. a. Glutinous corn starch. b. Tapioca starch. c. Corn starch. ×300.

starch. The degree of stretch of the pasted granules was, however, the same as for the untreated starch. Treatment of corn starch with dilute solution of either acid or alkali did not alter its potentiality for being stretched.

COHESIVENESS OF STARCH GRANULES

Sulfur dioxide treatment had a marked effect on potato starch granule properties. Potato starch processed with water (No. 6, table I) was very difficult to handle with a micromanipulator because of the stickiness of the

gelatinized granules and their tendency to adhere to the cover slip. When a sample of this starch was washed with 0.2 per cent. SO_2 solution, dried at 40° C. and subsequently gelatinized and stretched, there was no tendency for the granules to adhere to the cover slip. The treatment also lowered the paste viscosity.

Micromanipulative tests were made on 3, 4, and 5 per cent. pastes of commercial corn starch to determine how easily granules could be separated from the main body of the paste. It was very easy to disengage granules from the 3 per cent. paste. Whenever 2 or 3 granules pulled away together they could be readily separated from one another. In the case of the 4 per cent. paste, it was somewhat harder to separate granules from the paste and from each other than in the 3 per cent. sample. When a microneedle was fastened onto a granule near the edge of the 5 per cent. paste and a pull exerted, a large piece of the paste would separate from the main portion of the sample. It was very difficult to disengage individual granules from this gel.

These samples were again examined after having stood overnight at about 5° C. In every instance it was more difficult to separate the granules than it had been when the freshly prepared material was used. It was also more difficult to engage a granule with the microneedles after the paste had stood overnight, as the granules became hard and rubbery. Reheating the paste to 100° C. did not facilitate the separation of an individual granule from the paste nor was it easier to fasten a microneedle onto a granule. The changes which had taken place during refrigeration were therefore not reversible.

Similar tests were made upon potato starch pastes. The 3 per cent. paste was very elastic and although the granules would undergo considerable stretch before pulling away from the paste, individual granules could be separated fairly easily. The 4 per cent. paste was very cohesive but individual granules could be pulled away from the main body of the paste. The 4 per cent. and 5 per cent. pastes were almost identical.

Summary

1. A micromanipulative study was made of starches, including corn, glutinous corn, tapioca, and potato to determine whether length of paste might be correlated with ability of the individual granules to stretch without breaking. The data show that there is no apparent correlation between these two phenomena.

2. Starch granules pasted at 100° C. can be stretched to a greater extent than similar granules pasted at 80° C. for the same length of time.

3. When identical corn starch samples were pasted at a given temperature for 10, 30, and 60 minutes, and 72 hours, a significantly greater stretch was exhibited by the samples pasted for 30 minutes than for the others, all of which were essentially alike in ability to stretch.

4. Pretreatment of starch granules with 0.2 per cent. SO_2 or N/32 NaOH did not cause any appreciable change in the percentage of stretch of the

granules. These treatments respectively decreased and increased the paste viscosity.

5. Stretching the gelatinized granules did not bring about realignment of the molecules sufficient to cause a return of birefringence.

6. Individual granules could be pulled away from the main body of a 3 per cent. corn starch paste with ease. Potato starch granules would undergo more stretch than corn starch granules before separating but they, too, came away with ease. It was difficult, however, to separate single granules from a 4 or 5 per cent. corn or potato starch gel.

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THE EFFECT OF LIGHT ON THE ELECTRICAL POLARITY AND THE RATE OF ELONGATION OF THE AVENA COLEOPTILE[1]

A. R. SCHRANK

(WITH THREE FIGURES)

Introduction

Illumination of plants causes their electrical polarity and growth rate to change. These facts were observed years ago, but the relationship, if any, between these two changes has not been established. The experiments to be presented are concerned with this relationship.

It appears that HAAKE (**9**) was the first investigator to observe alterations in the electrical polarities of plants caused by changes in illumination. WALLER (**16**), who cites the literature to 1925, presented evidence for associating the electrical changes of green leaves with the activity of chlorophyll. The etiolated seedlings used by him gave little if any electrical response when illuminated. Since 1925, GLASS (**8**) and BROWN (**5**) have shown that the electrical polarity of Elodea leaves and internodes of *Chara vulgaris* manifest notable changes when their illumination is varied. SHEARD (**12**) exposed leaves of the sunflower and Poinsettia to both ultra-violet and infra-red light and obtained potential changes as high as 0.3 volt. Effects of light on the bioelectric potentials of Halicystis and Valonia have also been noted (**2, 10, 11**). It happens, however, that the effect of radiant energy on the bioelectrical potentials of etiolated plants has rarely been observed. CLARK (**6**) uniformly illuminated 30- to 40-mm. etiolated Avena seedlings with 600,000 meter-candle-seconds of incandescent light and recorded changes in the longitudinal electrical polarity. His data show a diphasic electrical variation in which the polarity first decreases, then increases before returning to the original value.

Numerous experiments have been performed to show the influence of illumination on the growth of the Avena coleoptile. One aspect of this problem deals with the *inhibition* of growth (**3**). VAN DILLEWIJN (**7**) subjected the Avena coleoptile to uniform illumination and noted effects on the growth rates by both continued and short-period irradiation. After a latent period, he observed a period of depressed growth showing two types of responses. In the first and short response, the maximum growth-rate depression was reached after 30 minutes. The second and longer reaction reached the maximum growth-rate depression after 90 minutes. It has been demonstrated that the second depression can be detected only when the apex of the plant is illuminated. [WENT (**17**): tip response.] The short period of depressed growth rate can be observed when either the basal portion or the entire coleoptile is illuminated. (WENT: base response.) The present ex-

[1] Supported by the University of Texas Research Institute.

periments are concerned with the first short period of growth rate depression and the corresponding electrical polarity changes.

Materials and methods

Coleoptiles of *Avena sativa*, Victory strain (C.I. 2020, obtained from the U. S. Department of Agriculture) were used in all experiments. The procedure for sprouting and growing the seedlings has previously been described (**14**). Sheaths 30 mm. in length were isolated from 35-mm., actively growing, straight coleoptiles and placed in a glass holder. This holder is shown in the inset of figure 2. The sheath in the holder was always placed in the apparatus (**13**) with the electrical contacts in position and allowed to remain undisturbed for 2 hours before beginning the experiment. One contact was placed at the apex and the second 15 millimeters basal to the first.

A 100-watt, frosted General Electric mazda lamp was used as the light source. This lamp was housed in a cylindrical reflector 18 cm. long and 11 cm. in diameter. The reflector was so placed that the open end of the cylinder was 40 cm. directly above the apex of the coleoptile. Heat from the lamp was absorbed by a layer of water 2 cm. deep located 15 cm. below the lower rim of the reflector. This arrangement gave a light intensity of 90 foot-candles at the position of the coleoptile. The light intensity measurements were made with a Model 603 Weston Illumination Meter.

The entire length of the sheath was illuminated by placing an inverted (larger base upward) truncated cone underneath it. This cone was prepared by cutting the stem from a 50-mm. glass filtering funnel and silvering it by the Brashear process. The position of the holder was always adjusted to place the basal end of the vertically oriented coleoptile in the center of the larger and upward base of the cone. The light intensity was measured at 5 mm. intervals along the longitudinal axis of the coleoptile position by a single junction thermocouple connected to a galvanometer. This thermocouple with its blackened 2×4 mm. receiver, all enclosed in a glass bulb, was held in position and moved by a micromanipulator. The galvanometer readings show the light to be 6 per cent. more intense at the apex than at the base of the sheath.

All experiments were performed at a temperature of 23 ($\pm$ 1.5) degrees centigrade in a dark room under a neon light constructed out of ruby glass tubing. The electrical measurements were made with a DuBridge (**4**) vacuum tube voltmeter using isoelectric ($\pm$ 0.5 mv.) zinc–zinc sulphate electrodes. Glass rings were used as the contacts with Shive's solution in tap water as the contact medium. Elongation was recorded with an ocular micrometer.

Experimental data

Effect of a 20-minute illumination period on the isolated sheath

All experiments were started at the end of the two-hour rest periods.

Longitudinal E.M.F.'s, temperature, and elongation readings were started and taken at 5-minute intervals during the remainder of each experiment. Thirty minutes after the beginning of each experiment the light above the coleoptile was turned on and left on for an arbitrary period of 20 minutes. This period of illumination is indicated in figure 1A by the heavy bar below the base line of the E.M.F. curves.

Curves I and II in figure 1A show the changes in the longitudinal electrical polarity of the apical 15 mm. of an isolated coleoptile when illuminated for 20 minutes. These two curves are from duplicate experiments. They are both included because they represent the two typical types of longi-

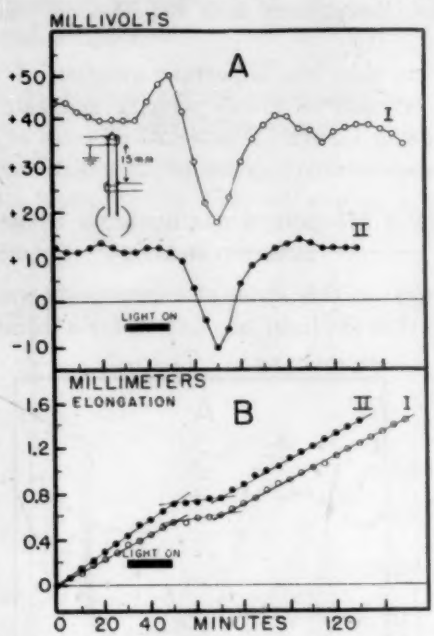

FIG. 1. Effects of a 20-minute illumination period on 30-millimeter, isolated coleoptile sheaths. A. Changes in the longitudinal electrical polarities of the apical 15 millimeters of two sheaths. B. Elongation rates of the same coleoptiles.

tudinal electrical polarity changes in the 15 experiments that were performed. These two curves differ in two respects. First, curve II crosses and goes beneath the 0 millivolt line. This means that the electrical polarity of this coleoptile is inverted for a short period of time. At the present time, no significance can be attached to this inversion. Secondly, there is a small increase in the electrical polarity as shown in curve I while the light was still on. This was observed in about half of the experiments. The total polarity decrease shown by curve I is about the same as shown by curve II. Both E.M.F. curves return to their original value in 60 minutes after the light was turned on.

The elongation curves shown in figure 1B correspond to the E.M.F. curves of figure 1A. The original rates of elongation in 15 experiments

varied from 8 μ/min. to 11 μ/min., while the original electrical polarities of these same sheaths varied from 10 mv. in some to 40 mv. in others. Both of the elongation curves show a temporary decrease in the rate of elongation beginning 20 minutes after the light was turned on. All of the 15 experiments that were performed showed this phenomenon without exception. Furthermore, it will be noted that the period of the decreased elongation rate corresponds exactly to the period of decreasing polarity as shown by the curves in figure 1A. Every experiment that was performed duplicated this relationship. After the period of decreased elongation rate, an increased rate was always resumed. (See curves I and II in figure 1B.)

The temperature throughout each experiment was kept constant to within ± 0.25° C.

These experiments raise the important question of whether or not the resumption of the original electrical polarity and rate of elongation was due to the light's being turned off after 20 minutes of illumination. The data which follow answer this question in the negative.

Effect of a 110-minute illumination period on the isolated sheath

The procedure used in this series of experiments was identical with the previous one except that the light was left on for a duration of 110 minutes instead of 20. Results of a typical experiment on the isolated sheath are shown in figure 2.

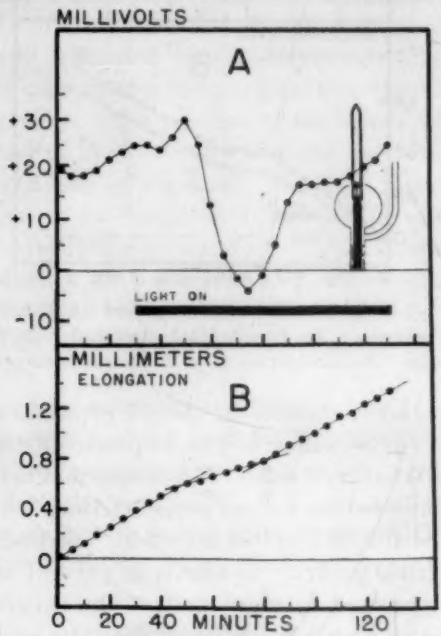

Fig. 2. Effect of continuous illumination on a 30-millimeter, isolated coleoptile sheath. A. Changes in the longitudinal electrical polarities of the apical 15 millimeters of one sheath. B. Elongation rates of the same coleoptile.

The E.M.F. curve shown in figure 2A is similar to the E.M.F. curves in figure 1A in all respects. The electrical polarity starts to decrease 20 minutes after the light is turned on, decreases for 25 minutes, and returns to the original polarity in 60 minutes. The elongation curve in figure 2B shows, as in previous experiments, a period of decreased rate of elongation which corresponds to the period of decreasing electrical polarity. (Compare figures 1B and 2B.) Duplicate experiments using intact 30-mm. plants corroborate these data in all aspects. These curves prove that the return to the original electrical polarity and the resumption of the normal growth rate are not dependent on turning the light off, and they further verify the fact that the period of decreased growth rate corresponds exactly to the period of decreasing electrical polarity.

Depression of elongation in apical and basal halves of the coleoptile

In these experiments intact 30-mm. (± 1 mm.) coleoptiles were used because they elongate more rapidly than isolated sheaths. An India ink

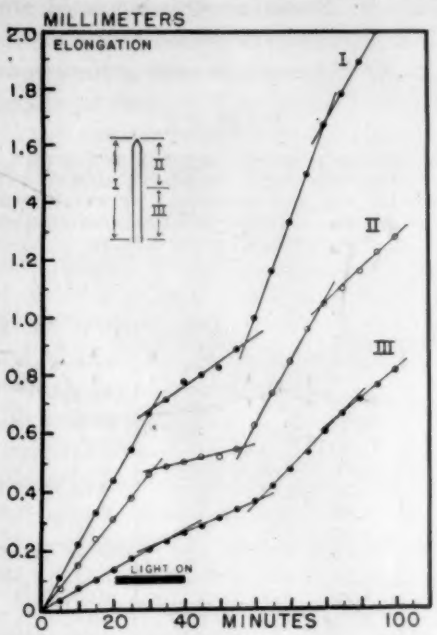

Fig. 3. Elongation rates of intact 30-millimeter Avena coleoptiles. I. Elongation of the complete plant. II. Elongation of the apical half. Obtained by subtracting points on curve III from corresponding points on curve I. III. Elongation of the basal half of the coleoptile.

spot was placed half way between the apex and base after which the plant was placed in the holder and allowed to remain undisturbed for 30 minutes. Each experiment was then started by recording the position of the apex and the ink spot by the use of ocular micrometers in horizontal microscopes.

These readings were repeated at 5-minute intervals and continued for 100 minutes. Twenty minutes after the beginning of the experiment the light was turned on and left on for an additional 20 minutes. Figure 3 shows the results of one of these experiments. Curve I represents the elongation of the complete coleoptile and was obtained by recording the position of the apex. Curve III, which shows the growth rate of the basal half, was plotted by following the position of the India ink spot. The growth rate of the apical half of the coleoptile is shown by curve II which was obtained by subtracting the points on curve III from the corresponding points on curve I.

The curves in figure 3 show that incandescent light of 90 foot-candles for 20 minutes causes a temporary decrease of the growth rate of both the apical and basal halves of the Avena coleoptile. Comparison of curves II and III indicates that the growth rate in the apical half is more effectively depressed than it is in the basal half. A duplicate series of 10 experiments using the isolated coleoptile sheaths verified these facts. Further inspection of the curves shows that the rate of elongation which is resumed after inhibition is greater in every curve than the original rate. This fact was observed in all experiments. Sometimes this increased growth rate results in a net increase in growth during the period of the experiment as shown by curve III. Why this should occur in some instances and not in others is

TABLE I

ELONGATION RATES EXPRESSED AS SLOPES OF LINES OF 8 INTACT AVENA COLEOPTILES, SHOWING ORIGINAL GROWTH RATES, DECREASED GROWTH RATES CAUSED BY ILLUMINATION, AND THE DIFFERENCE IN THESE TWO RATES. A SLOPE OF 1 IS EQUAL TO AN ELONGATION RATE OF 10 MICRONS PER MINUTE

	A			B			C		
	COMPLETE COLEOPTILE*			BASAL HALF†			APICAL HALF‡		
EXP. NUMBER	ORIGINAL RATE	DECREASED RATE	DIFFERENCE	ORIGINAL RATE	DECREASED RATE	DIFFERENCE	ORIGINAL RATE	DECREASED RATE	DIFFERENCE
1	2.20	0.80	1.40	0.68	0.53	0.15	1.52	0.27	1.25
2	1.60	0.87	0.73	0.48	0.33	0.15	1.12	0.54	0.58
3	1.82	0.93	0.89	0.50	0.33	0.17	1.32	0.60	0.72
4	2.20	0.93	1.27	0.40	0.33	0.07	1.80	0.60	1.20
5	1.54	0.87	0.67	0.34	0.27	0.07	1.20	0.60	0.60
6	1.90	0.60	1.30	0.30	0.13	0.17	1.60	0.47	1.13
7	2.18	1.07	1.11	0.58	0.33	0.25	1.60	0.74	0.86
8	1.50	0.73	0.77	0.46	0.27	0.19	1.04	0.46	0.58
Av.	1.87	0.850	1.01	0.467	0.315	0.153	1.40	0.534	0.866

* Slopes obtained graphically from plotted lines.
† Same coleoptiles; slopes obtained graphically.
‡ Same coleoptiles; slopes obtained by subtracting slopes in B from slopes in A.

not clear. It will be noted that the period of decreased growth rate shown in figure 3 begins 10 minutes after the light was turned on. In all the other experiments 20 minutes elapsed after turning on the light before the decreased rate became apparent. The data in figure 3 were selected because

they represent all the experiments in this series in all other respects. Examination of the averages of the 8 duplicate experiments performed in this series reveals two other facts. The average duration of the period of decreased growth rate of the entire coleoptile and of the apical half is 19.5 minutes (varying from 16 to 26 minutes). In the basal half the period of inhibition is an average of 7.6 minutes longer. This longer period of inhibition is evident in curve III of figure 3.

The data in table I were taken from the 8 experiments in this group. All of the numbers represent slopes corresponding to the curves of figure 3. A slope of 1 is equal to an elongation rate of 10 microns per minute. Section A of the table comes from growth rates of the entire coleoptile corresponding to curve I. Similarity, section B was obtained from growth rate of the basal halves of the same plants shown in section A. Section C was obtained by subtracting the slopes in section B from the slopes in section A. These slopes correspond to curve II in figure 3. The slopes in the first column of each section represent the original elongation rates while the slopes in the second columns represent the decreased growth rates. The change in growth rates is shown in column three of each section. This column was obtained by subtracting the slopes representing the decreased rates from slopes representing the original rates. This table gives an indication of the uniformity of the experimental results, and the comparison of the average change in elongation rates show that the apical half of the coleoptile is responsible for about 85 per cent. of the growth inhibition.

Discussion

The changes in the longitudinal electrical polarity of the apical 15 millimeters of the isolated Avena coleoptile sheath shown by the data presented are quite similar to the changes recorded for intact plants by CLARK (**6**). Following the period of decreased polarity, Clark's curves show a period of increased polarity which was not manifested by the isolated sheaths. The data indicate that the isolated coleoptile (with the primary leaves removed) is capable of absorbing radiant energy and responding electrically. Minute quantities of carotenoid pigments, which might well be responsible for the photoreception, have been isolated from the Avena coleoptile (**15**).

The reversible depression of the growth rate of the isolated sheath as shown by the curves in figures 1B and 2B is in agreement with the observations of VAN DILLEWIJN (**7**). Since the cells in coleoptiles of the lengths that were used are no longer dividing (**1**), the depressed growth rate must be due to a *decreased rate of cellular elongation.* Whether this decreased rate of cellular elongation is caused by inactivation of the growth hormones, a decreased rate of auxin production, hindrance of hormone transport, or by a change in the reactivity of the cells to the hormones present remains to be demonstrated. The growth responses of the intact coleoptile differ from those of the isolated sheath in two aspects. The intact plant always manifests a period of increased growth rate (faster than the original growth

rate) following the period of decreased growth rate. (Compare figure 3 with the curves in figures 1B and 2B.) The growth rate of the intact plant is always somewhat faster than that of the isolated sheath.

Evidence that is available at the present time indicates that the changes in bioelectric and growth phenomena in the Avena coleoptile are related. (1) Electrical and elongation rates change simultaneously. In the dozens of experiments that were performed on both isolated sheaths and intact plants, the period of decreasing electrical polarity is always accompanied by a depressed growth rate. (Figures 1 and 2.) (2) Isolated coleoptiles do not show a period of increased elongation following the period of decreased elongation. Neither do the electrical polarities show a period of increased polarity following the period of decreased polarity. The intact plants show both of these phenomena. (See figure 3 and CLARK (**6**).) (3) Turning off the light affects neither the electrical polarity nor the growth rate. (Compare figures 1 and 2.) (4) Data from table I show that the apical half of the coleoptile is responsible for about 85 per cent. of the growth rate depression. CLARK'S (**6**) curves demonstrate that the light-induced electrical polarity changes are practically limited to the apical half of the plant. It is possible that these changes in electrical polarity and growth rates, which are caused by illumination, could be parallel but independent effects. The facts just presented make this seem unlikely. What the linkage is between the decreased growth rate and the decrease in electrical polarity remains to be determined.

Summary

1. The apical half of a 30-mm. isolated Avena coleoptile sheath responds to uniform illumination by changing its electrical polarity. After a delay of 20 minutes the polarity decreases by 20 to 25 mv. and then returns to the original value.

2. The period of decreasing electrical polarity is always accompanied by a decreased rate of elongation.

3. Continuous and short-period illumination result in similar electrical polarity and growth rate changes.

4. In the intact coleoptile the period of decreased growth rate, which averages 19.6 minutes in length, is always followed by an elongation rate greater than the original.

5. The apical half of the coleoptile is responsible for about 85 per cent. of the growth rate depression.

6. Evidence indicates that the changes in bioelectric phenomena and the changes in rates of elongation are linked.

The technical assistance of MISS GLENDA OGLESBY during this investigation is acknowledged with pleasure.

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SOME PHYSICAL AND CHEMICAL CHANGES IN THE COMPOSITION OF PECAN NUTS DURING KERNEL FILLING[1]

HAROLD E. HAMMAR AND JAMES H. HUNTER

(WITH NINE FIGURES)

Introduction

Considerable work has been done on the anatomy of the pecan nut and its physiological and morphological development (**4, 5, 8, 15, 21, 22, 23**). The most complete systematic investigation relative to the development of the fruit of the pecan from an early pre-filling stage to maturity, including changes in the oil, protein, and carbohydrate formation was reported by THOR and SMITH (**19, 20**) who also gave a comprehensive review of the literature relating to studies on the physiological development of the pecan nut, together with the oil, protein, and carbohydrate formation in the fruit and seeds of other plants. CRANE and HARDY (**3**) have reported on the effect of cultural and fertilizer treatment on the filling and chemical composition of the pecan, and concluded that the best filled nuts have the highest percentage of oil.

A number of investigators have reported on the analyses of the kernels of mature nuts (**3, 5, 12, 17, 19, 22**). SHUHART (**14**) stressed the important function of the pecan hull in the translocation of materials stored in the kernels. SMITH and LOUSTALOT (**16, 17**) studied the effect of the date of harvest and of curing on the composition of the pecan kernel, shuck, and shell and reported the oil, protein, and carbohydrate changes that took place. FINCH (**4**) stated that the rapid movement of food materials into the nuts in late summer arose chiefly from those stored elsewhere earlier in the summer.

Very little work has been reported on the mineral content of pecan nuts, especially in regard to the mineral changes taking place during kernel development. HUNTER and LEWIS (**7**) presented data indicating that the minerals from fertilizers applied at different times during the growing season were taken up and influenced the physiological processes of the tree, as evidenced by differences found in the degree of filling of the nuts at maturity. These observations led them to investigate further the chemical changes occurring in the mineral constituents of the nuts and their supporting shoots at four stages of development. They found (**11**) that at the period in which the kernel developed and filled, substantial quantities of nitrogen, potassium, magnesium, and phosphorus accumulated in the nut, while the supporting shoots lost significant quantities of all these elements.

Recent studies by the authors (**6**) showed that a surprisingly large amount of potassium accumulated in the shucks and that there were other

[1] This paper was presented before the Georgia Section of the American Chemical Society held in Atlanta, September 28, 1945.

important changes in the nuts during development. These results stimulated further study of the nitrogen and the mineral constituents of the various parts of the nut during kernel development to determine the part each played, and their effect on the quality of the mature nut.

In a pecan orchard near Albany, Georgia, thirty nuts were picked from clusters of four nuts from each of three trees of the Moore variety at weekly intervals from August 25, 1944, when the kernels were in an early filling (late jell) stage, to full maturity on October 27, 1944. The same trees were used for each sampling, and duplicate determinations were made on the combined sample of thirty nuts from each tree for each of the nine sampling dates.

The shucks were removed, and the nuts were allowed to dry several days in a low temperature oven, after which they were weighed. The shuck samples were dried in an oven at 60° C. and the oven-dry weight was determined. The shucks from the nuts taken on the first three sampling dates had to be removed by cutting and scraping the material away from the shell with a sharp knife, while those from later sampling dates had separated themselves from the nuts. After drying, the nuts were cracked, the kernels were separated from the shells, and the air-dry weights of each were determined.

In preparing the kernel sample for analysis, it was ground in a home-made slicer to pass a 10-mesh sieve and moisture was determined by drying in a vacuum oven at 60° C. for 15 hours at a reduced pressure of 25 inches of mercury. Oil was determined on the air-dry sample using the sulphuric-acid digestion method of LEWIS (**10**). The shuck and shell samples were ground in a Wiley mill to pass a 60-mesh sieve. One-gram samples of oven-dry kernels and shucks were used for total nitrogen (and protein) determinations, and 5 grams for the determination of ash from which phosphorus, potassium, calcium, and magnesium were determined. Two-gram samples of the oven-dry shell were used for nitrogen determinations, and 10 grams of the material were used for ash and the mineral constituents.

The samples were ashed first under low heat, and finally for 2 hours in an electric muffle at 550° C. Official methods of the A.O.A.C. (**1**) were used for all the analyses except for potassium, in which the sodium-cobaltinitrite method of BROWN, ROBINSON, and BROWNING (**2**) was used. Duplicate determinations were made on each sample. Protein in the kernel was calculated by multiplying the total nitrogen by 5.3 (**9**). Sugars and acid-hydrolyzable polysaccharides were not determined, as sufficient data on these constituents had been determined and discussed in detail by previous investigators (**5, 17, 19, 20**).

Results

The data for the mean chemical composition calculated on the percentage of oven-dry weights for the kernel, shuck, and shell materials, and on each of the nine sampling dates, are given in table I. The same data, but calculated on a unit basis of milligrams of constituents per nut, are given in table II.

The data are expressed on the percentage and unit basis because of the striking differences in the curves representing the data obtained for the kernel, and the similarity in the curves representing the data on the shucks and shells of the nuts during development. The data expressed on the unit basis probably show the changes taking place more clearly than when they are expressed on the percentage basis.

TABLE I

MEAN CHEMICAL COMPOSITION OF PECAN NUTS
PERCENTAGE BASIS

DATE	NITROGEN	PHOSPHORUS	POTASSIUM	CALCIUM	MAGNESIUM	ASH	OIL	PROTEIN
	KERNELS							
1944	%	%	%	%	%	%	%	%
8/25	2.00	0.379	1.228	0.139	0.180	4.56	51.5	10.62
9/1	1.89	0.340	0.686	0.114	0.150	2.81	66.8	10.02
9/8	1.96	0.335	0.441	0.089	0.132	2.48	70.8	10.38
9/15	2.03	0.342	0.405	0.078	0.118	2.50	72.3	10.78
9/22	1.99	0.328	0.399	0.077	0.112	2.19	73.8	10.53
9/29	2.00	0.335	0.428	0.072	0.128	2.41	73.0	10.60
10/6	2.01	0.334	0.427	0.069	0.128	3.15	72.3	10.65
10/13	2.02	0.369	0.448	0.078	0.104	2.65	72.5	10.70
10/27	1.81	0.360	0.394	0.085	0.097	2.47	74.4	9.57
	SHUCKS							
8/25	1.35	0.239	2.925	0.790	0.279	9.04		
9/1	1.25	0.219	3.303	0.825	0.293	10.45		
9/8	1.16	0.221	4.377	0.703	0.287	12.45		
9/15	1.09	0.215	4.908	0.637	0.306	13.38		
9/22	1.04	0.206	4.986	0.663	0.291	12.30		
9/29	1.06	0.207	5.075	0.700	0.289	12.61		
10/6	0.99	0.211	4.955	0.795	0.305	13.07		
10/13	1.04	0.202	4.706	0.633	0.294	12.39		
10/27	0.97	0.180	4.022	0.961	0.332	11.87		
	SHELLS							
8/25	0.290	0.029	0.163	0.560	0.077	2.26		
9/1	0.282	0.022	0.172	0.559	0.038	2.00		
9/8	0.292	0.020	0.172	0.585	0.012	2.00		
9/15	0.334	0.023	0.170	0.550	0.045	2.06		
9/22	0.318	0.022	0.207	0.541	0.039	2.20		
9/29	0.323	0.020	0.221	0.532	0.031	2.48		
10/6	0.315	0.029	0.211	0.603	0.052	2.26		
10/13	0.320	0.032	0.198	0.540	0.065	2.03		
10/27	0.294	0.026	0.193	0.592	0.040	2.24		

It should be noted that the first sampling date on August 25 did not represent the initial stage of development of the kernel, since over 22 per cent. of the dry weight had been formed prior to the first sampling period, but it does represent the early filling stage.

CHEMICAL CHANGES IN THE KERNEL

The oil, protein, dry weight, and ash of the kernel are graphically shown in figure 1 to increase very rapidly during the first three weekly sampling

periods. Similar changes have been shown by other investigators (**11, 19, 20**). Approximately 96 per cent. of the protein, 82 per cent. of the oil, 85 per cent. of the dry weight, and 84 per cent. of the ash of the kernel at maturity date were formed prior to the fourth sampling date. An indication of the rapid change that takes place in the kernels is that the data show 63 per cent. of the total dry weight, 64 per cent. of the total oil, 43 per cent.

TABLE II

MEAN CHEMICAL COMPOSITION OF PECAN NUTS
UNIT BASIS

DATE	WT. PER NUT	NITROGEN	PHOSPHORUS	POTASSIUM	CALCIUM	MAGNESIUM	ASH	OIL
				KERNELS				
1944	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
8/25	0.556	11.23	2.11	6.69	0.80	0.95	24.04	350.2
9/1	1.151	21.80	3.87	7.80	1.35	1.72	33.80	772.2
9/8	1.667	32.27	5.50	7.13	1.51	2.24	40.31	1184.5
9/15	2.110	42.96	7.17	8.57	1.70	2.46	49.93	1526.8
9/22	2.370	44.78	7.48	9.13	1.80	2.46	46.22	1703.6
9/29	2.384	47.11	8.05	10.20	1.74	3.09	62.63	1741.5
10/6	2.183	42.70	7.20	9.27	1.59	2.80	70.00	1577.9
10/13	2.296	45.67	8.37	10.07	1.93	2.66	61.05	1665.1
10/27	2.482	44.47	8.84	9.82	2.09	2.42	59.55	1845.2
				SHUCKS				
8/25	1.727	23.30	4.13	50.51	13.64	4.82	156.12	
9/1	1.723	21.60	3.77	56.91	14.21	5.05	180.05	
9/8	1.710	19.84	3.78	74.85	12.02	4.91	212.90	
9/15	1.743	18.95	3.75	85.55	11.10	5.33	233.32	
9/22	1.743	18.21	3.59	86.91	11.56	5.07	214.39	
9/29	1.793	19.04	3.71	90.99	12.55	5.18	226.10	
10/6	1.723	17.13	3.64	85.37	13.70	5.26	225.20	
10/13	1.753	18.16	3.61	82.50	11.10	5.15	217.20	
10/27	1.876	18.22	3.38	75.45	18.02	6.23	222.68	
				SHELLS				
8/25	2.324	6.74	0.67	3.79	13.01	1.79	52.52	
9/1	2.351	6.63	0.52	4.04	13.14	0.89	47.01	
9/8	2.324	6.79	0.47	4.00	13.60	0.28	46.49	
9/15	2.458	8.21	0.57	4.18	13.52	1.11	50.65	
9/22	2.330	7.41	0.51	4.82	12.61	0.91	51.27	
9/29	2.317	7.48	0.46	5.12	12.33	0.72	57.46	
10/6	2.156	6.79	0.63	4.55	13.00	1.12	48.72	
10/13	2.224	7.12	0.71	4.40	12.01	1.45	45.14	
10/27	2.471	7.26	0.64	4.77	14.63	0.99	55.35	

of the total ash, and 71 per cent. of the total protein to have been formed in the three-week period August 25 to September 15. The percentage of oil (figure 2) paralleled that expressed on a unit basis. The percentage of protein, however, followed a reverse curve to that expressed on a unit basis, by falling sharply during the first and the final stages of kernel development.

The decrease in the percentage of ash of the kernel, while the actual

weight increased when expressed on a unit basis, was no doubt due primarily to the rapid formation of oil, protein, and possibly carbohydrates, and not due to any significant loss of mineral constituents as will be shown. The content of ash and dry weight of the kernel was found to rise perceptibly the first few sampling periods and then to follow a gradual rise to maturity date. The phosphorus content on a unit basis as shown in figure 3 followed closely the curves given in the preceding charts for protein in the kernel, by

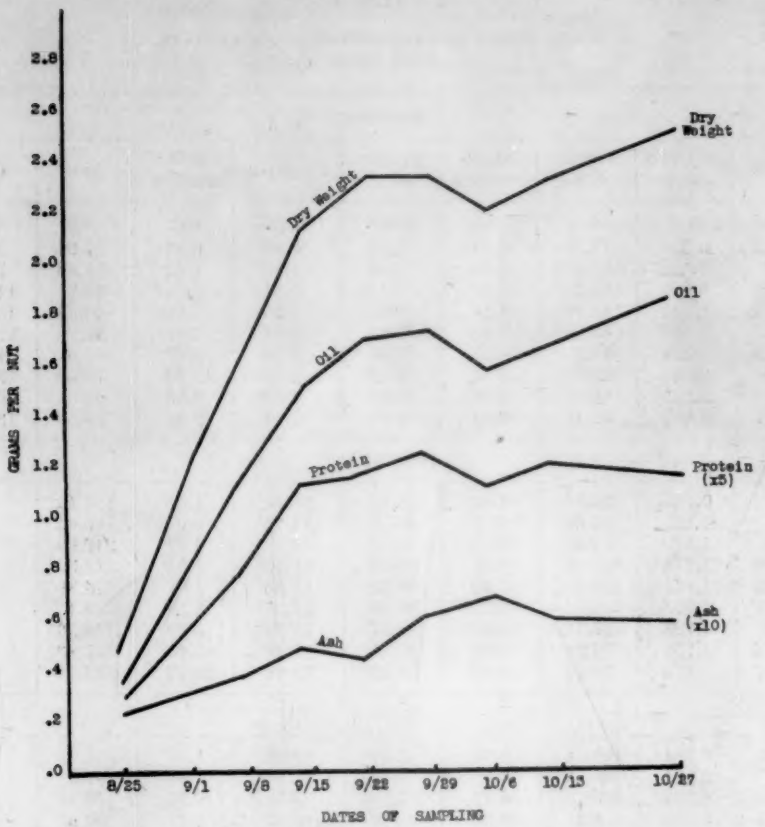

FIG. 1. Changes in dry weight, oil, protein, and ash in pecan kernels during filling. (Unit basis of grams per nut.)

rising sharply the first three weeks of sampling, and then gradually increasing in amount to maturity; while the potassium and magnesium rose more gradually to the mid-period of filling, and then declined slightly to the final stage. The percentages of phosphorus, magnesium, and calcium as shown in figure 4 remained practically the same throughout the period of filling, while that for potassium declined very sharply the first two weeks of sampling, and then leveled off to maturity. Magnesium and calcium were found to occur in the smallest amounts of the several mineral constituents in the pecan kernel.

Chemical changes in the shuck and shell

The content of all the mineral elements determined in the shuck and shell follow very similar curves, whether expressed as percentage or on a unit basis, as shown in figures 5, 6, 7, and 8.

The potassium content of the shuck was by far the greatest of all the mineral elements contained in the pecan nut, as shown in tables I and II. It comprised approximately 73 per cent. of the mineral elements determined.

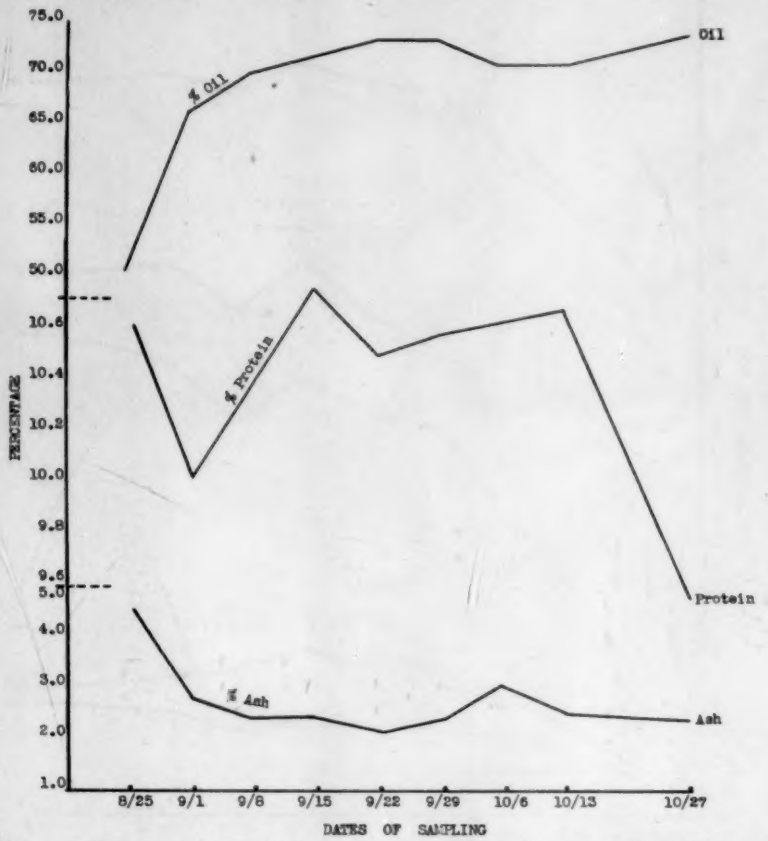

Fig. 2. Changes in oil, protein, and ash in pecan kernels during filling. (Percentage basis.)

and 34 per cent. of the total weight of the ash in the shuck. The potassium content rose rapidly in the shuck until the mid-period of filling was reached, and then declined gradually to the maturity date.

The calcium content of the shuck and shell followed somewhat similar curves, both rising sharply at the final stage of maturity. The curves for magnesium in the shell show a different picture. There was a sharp drop in magnesium in the shell during the first two weeks of sampling, and again at the final period, whereas the content of calcium increased sharply during the last two weeks. Calcium comprised more than half of the weight of the

mineral elements determined in the shell. Magnesium and phosphorus were contained in the least amounts in the shuck and shell.

Changes in the ash and dry weight of kernel, shuck and shell

Comparisons of the dry weight and ash in the kernel, shuck and shell of the pecan are shown in figure 9. The dry weight and the weight of ash in the kernel followed parallel curves; while, calculated as percentage, the ash

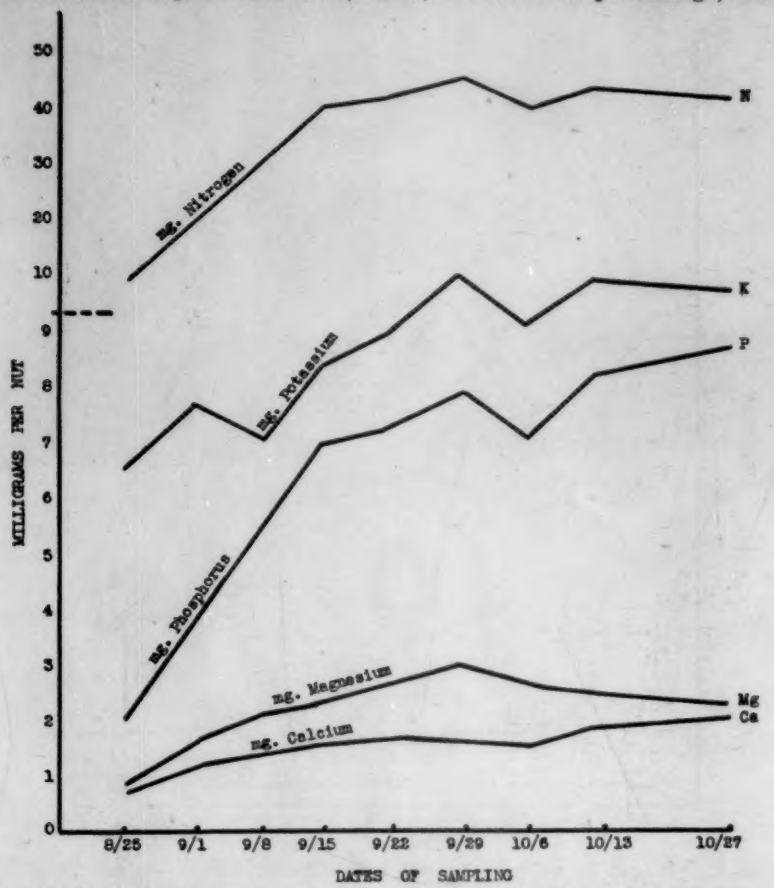

Fig. 3. Changes in nitrogen, phosphorus, potassium, calcium, and magnesium in pecan kernels during filling. (Basis of milligrams per unit.)

followed a reverse curve. However, the ash content of the shuck and shell was found to follow almost identical curves when expressed on percentage and on unit bases. The dry weight of the kernel was found to rise sharply during the early period of filling, while that of the shuck and shell rose rapidly during the final stage of filling.

It is interesting to note (table II) that much of the dry weight and ash of the shuck and shell had already developed at the beginning of the sampling period, while only a small amount of the dry weight and ash of the

kernel was present at that time. It was found that 92.1 per cent. of the dry weight of the shuck at maturity, and 94.1 per cent. of the shell, had already been formed at the first sampling period, while only 22.4 per cent. was found to have developed in the kernel. Likewise, 70.1 per cent. of the ash in the shuck at maturity, and 94.9 per cent. of the ash in the shell, had been deposited during the stage prior to filling, while only 40.4 per cent. of the ash in the kernel had been deposited at that time.

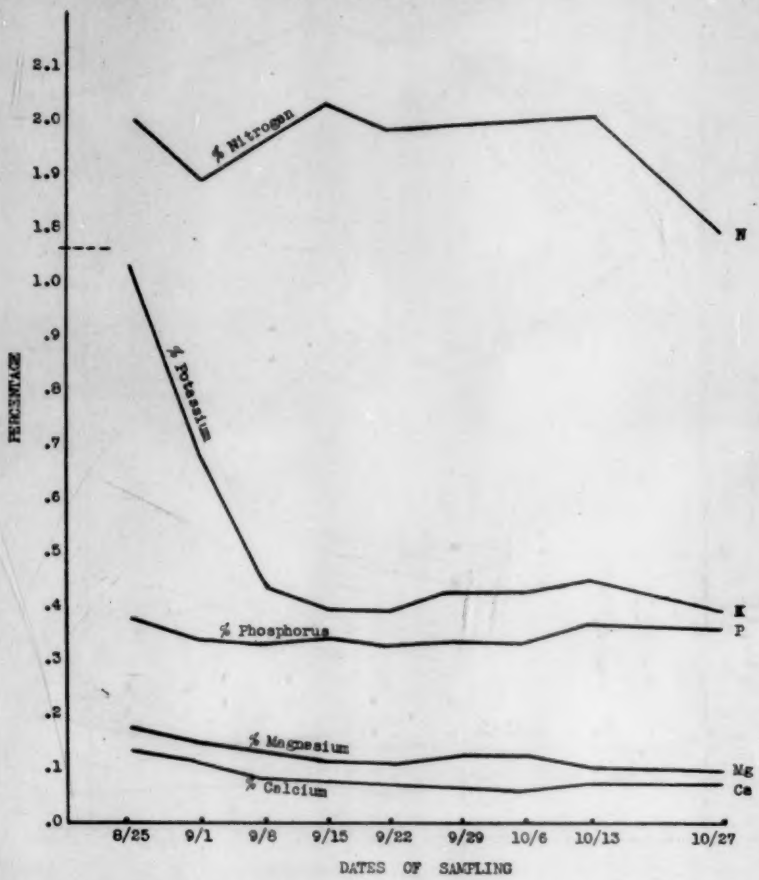

FIG. 4. Changes in nitrogen, phosphorus, potassium, calcium, and magnesium in pecan kernels during filling. (Percentage basis.)

Discussion

The results of oil, protein, and ash determinations in the kernel, shuck, and shell of the pecan agree closely with those found by other investigators (**17, 19, 20**). The data obtained by THOR and SMITH (**19, 20**) show that sugars are accumulated at a rapid rate in the whole fruit and shuck of the pecan during the pre-filling stage, falling rapidly soon after filling begins, and that during this pre-filling period much of the structural material has

been formed in the shuck and shell. They found that the development of the shell is practically complete at the time filling of the nuts begins, and this was borne out by ROMBERG *et al.* (**13**). The authors found this to be true since most of the dry weight and ash of the shuck and shell had accumulated at this time, whereas only a small portion of the weight of the kernel was evident. This bears out the conclusion of SMITH and THOR (**18**) that the first period of growth—the pre-filling period, prior to late August—

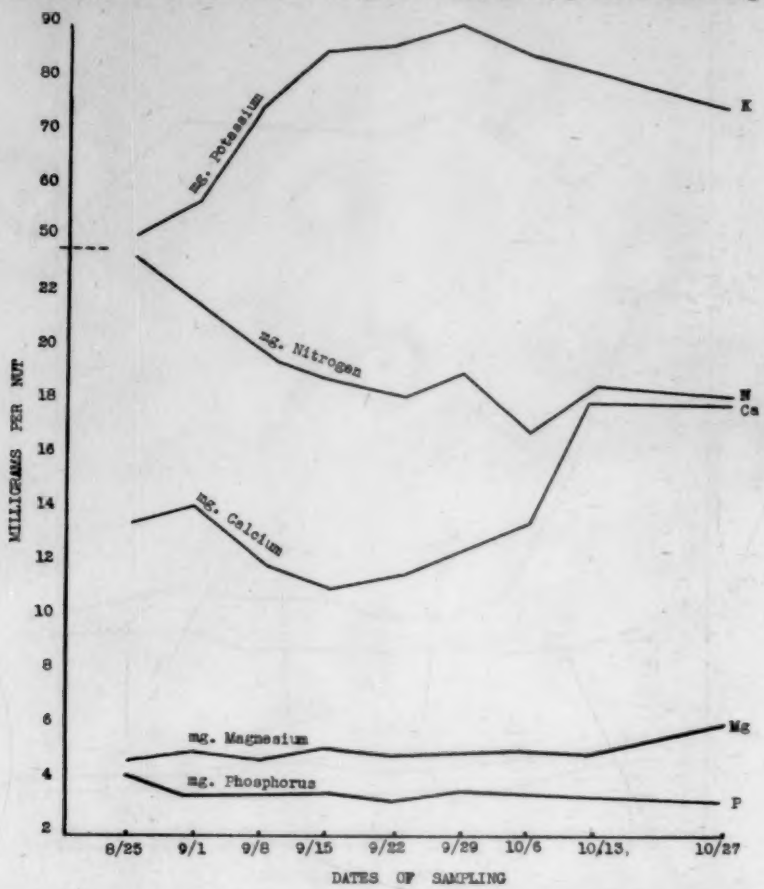

FIG. 5. Changes in nitrogen, phosphorus, potassium, calcium, and magnesium in pecan shucks during kernel filling. (Basis of milligrams per nut.)

is characterized by the formation of structural elements of the shuck and shell. During the second period, the filling of the kernel is of major importance; *i.e.*, formation of oil, protein, minerals, and acid-hydrolyzable polysaccharides. Therefore, it appears that the most important period in the development of the shuck and shell is the pre-filling period.

In the present investigation, most of the oil, protein, and mineral constituents of the kernel were found to be deposited preceding and during the

first three of the weekly periods of sampling, or before September 15, and this no doubt is the critical period in the development of the kernel.

FINCH (4) noted that by August 26, "filling" was occurring rapidly and deposition of solid materials continued for the ensuing 10 days, when the kernels were filled solidly. The marked differences obtained by calculating the data obtained during the filling of the kernel on the percentage basis and on the basis of weight per nut illustrate the important changes

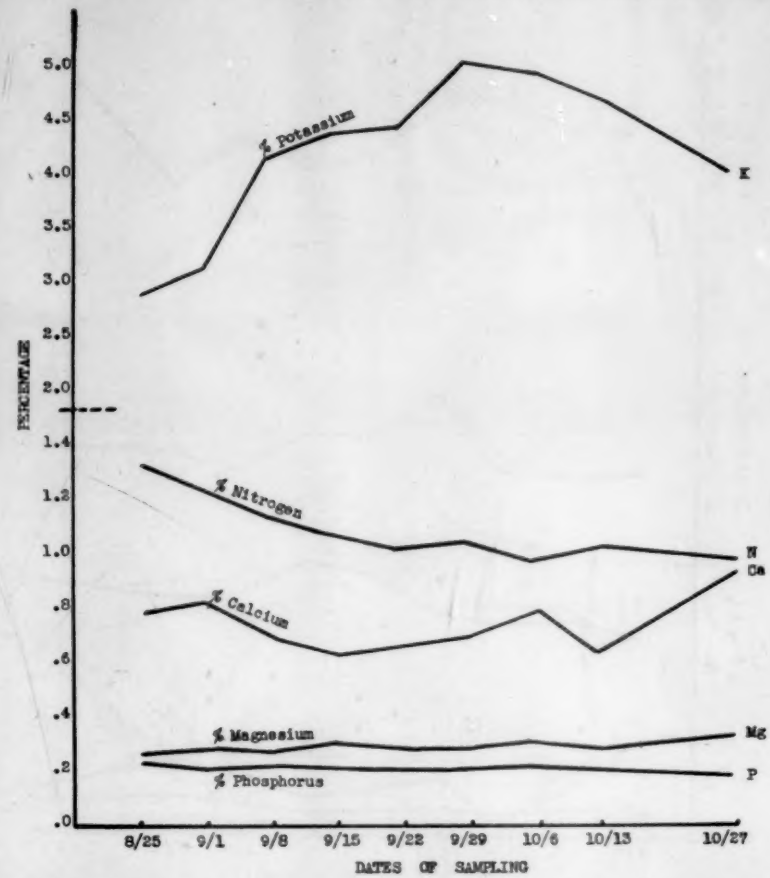

FIG. 6. Changes in nitrogen, phosphorus, potassium, calcium, and magnesium in pecan shucks during kernel filling. (Percentage basis.)

in the basic materials used in the development of the different portions of the pecan nut. The rapid formation of oil, protein, and carbohydrates in the kernel was accompanied by a similar rise in the mineral elements as shown in figure 3, but the amounts of these mineral elements were by far smaller than the amounts of the organic materials. Thus the decrease in the percentage of the ash constituents did not indicate any actual loss of these elements. On the other hand, the curves obtained by calculating the data

on percentage and on unit bases in the pecan shuck and shell were very similar, indicating that the change of materials was mainly mineral.

The changes in the chemical composition of the shuck are of utmost importance, since all the substances that enter the nut must pass through it before they can be formed in the kernel. A considerable portion of the mineral constituents of the pecan was contained in the shuck. Approximately 70 per cent. of the minerals (phosphorus, potassium, calcium, and

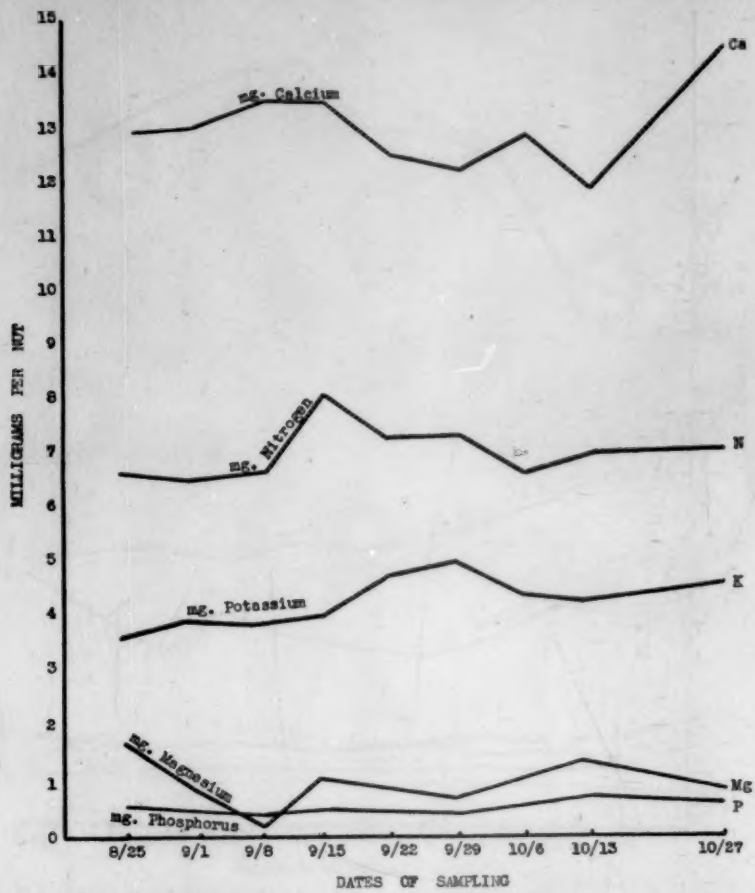

FIG. 7. Changes in nitrogen, phosphorus, potassium, calcium, and magnesium in pecan shells during kernel filling. (Basis of milligrams per nut.)

magnesium) of the mature pecan nut was contained in the shuck, while 14 per cent. was present in the shells, and 16 per cent. in the kernels.

At the time of the first sampling when the kernels were beginning to fill, the shucks contained approximately 71 per cent. of the mineral constituents, while the shells contained 19 per cent. and the kernels only 10 per cent. Thus it would seem that these chemicals perform a vital function in the filling of the kernel, either by aiding in the translocation of the materials

which go into the formation of the kernel, or directly as catalysts in the transformation into oil of carbohydrate material previously stored in the supporting shoots. The total sugar content of the pecan kernel is not built up until after the formation of oil and other constituents has stopped; and according to THOR and SMITH (**18, 19, 20**) and SMITH and LOUSTALOT (**16, 17**), this translocation is mainly from the shuck, which loses considerable quantities of sugars during the later filling stage. At the first sampling

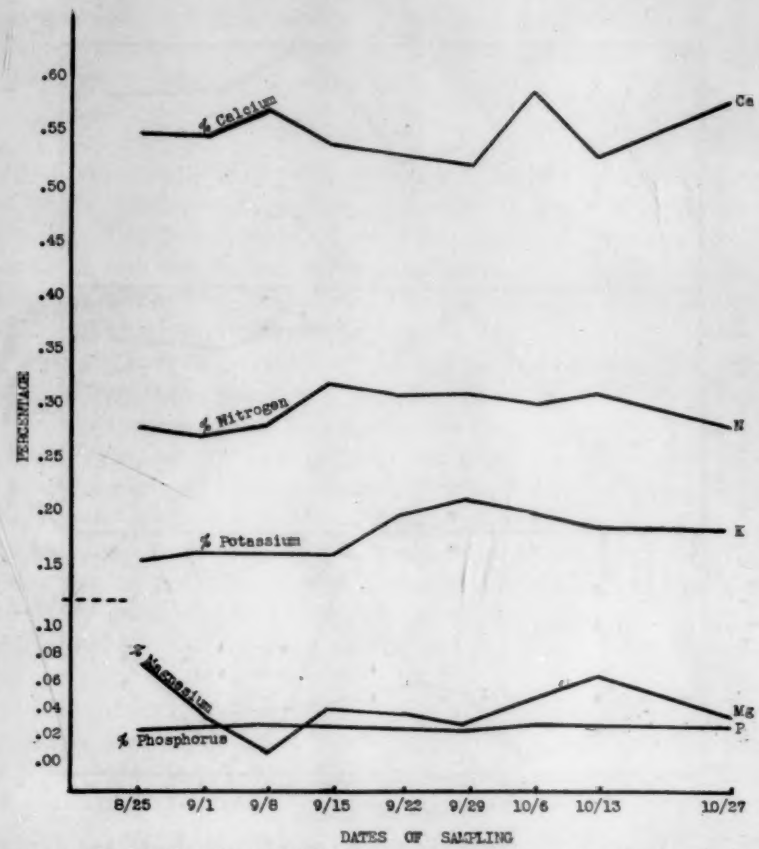

FIG. 8. Changes in nitrogen, phosphorus, potassium, calcium, and magnesium in pecan shells during kernel filling. (Percentage basis.)

period, approximately 92 per cent. of the dry weight of the shuck had been formed, but only 70 per cent. of the ash. This may be explained by the translocation of much of the sugars stored in the shucks at the beginning of the filling period, that material being translocated into the kernel during the later period of filling.

That potassium is very important in the filling processes in the pecan nut is evidenced by the large amounts present in the shuck, through which must pass all materials that go into the nut. The content of potassium increased

very rapidly in the shuck during the critical period of early filling. The gradual decline of the potassium content in the shuck after the mid-filling period had been reached indicated that potassium was transferred back into the supporting tissues, since there was no appreciable increase in the potassium content of either the shell or the kernel of the nut to account for the loss in the shuck. Another possible cause for the decline in the potassium content of the shuck during the later filling stage could have been leaching by rainfall. This, however, was very unlikely, as only 1.8 inches of rain

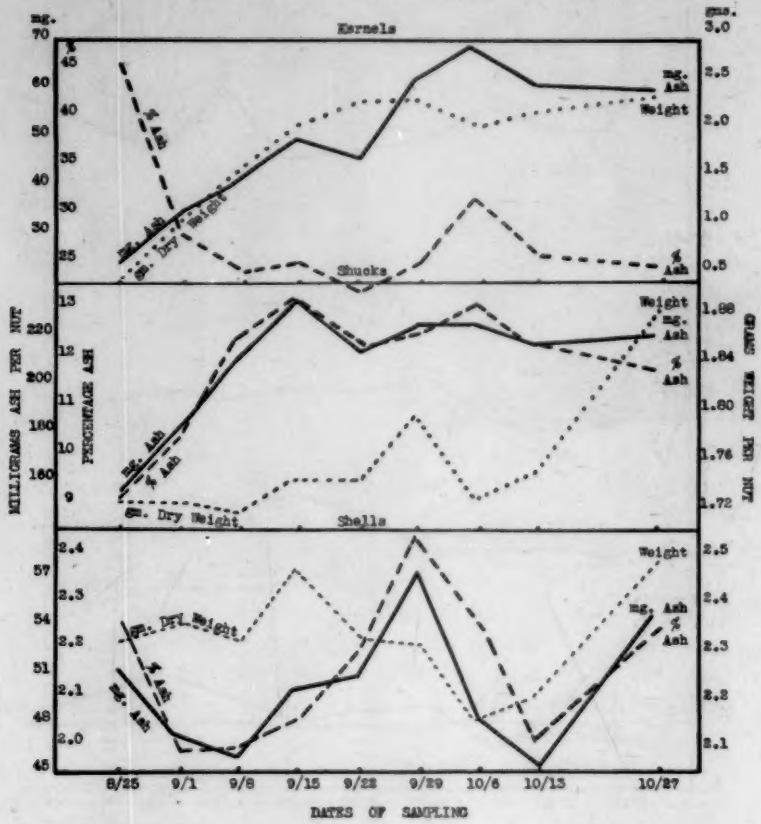

FIG. 9. Changes in dry weight and ash in pecan kernels, shucks, and shells during kernel filling. (Percentage and unit weight bases.)

were recorded in this orchard during the period in which this decline took place.

Phosphorus was found to increase rapidly in the kernel during the filling period, and no doubt performs an important function in the processes of filling. However, this element occupies only a minor position in the mineral content of the shuck and shell. The phosphorus content of the shuck declined steadily during the development of the kernel, which indicated that the filling process was drawing on phosphorus in the shuck. This,

however, would only account for about 10 per cent. of the phosphorus which went into the kernel during this period. Evidently this element was moved from the trees into the kernel with the assimilated products that go into kernel formation.

It appears that calcium is utilized mainly for the structural development of the shell, since it composed nearly 68 per cent. of the mineral constituents of the shell at the first sampling period. The shell of the pecan seems to have little or no function in the filling processes, since only minor chemical changes took place during kernel development. SMITH and LOUSTALOT (**16, 17**) did not find any appreciable change in any of the chemical constituents of pecan shells at different harvest dates.

Summary

A systematic sampling of pecan nuts was made at nine weekly intervals during kernel development to determine chemical changes in the mineral constituents. Dry weight, ash, oil, nitrogen (protein), phosphorus, potassium, calcium, and magnesium were determined on the kernels, shucks, and shells and the data were calculated and reported as percentage of dry weight and on the unit basis of milligrams per nut.

Striking differences occurred in the curves representing the data expressed on percentage and on unit bases for the constituents of the kernels, but there was a similarity in such curves for the constituents of the shuck and shell. This was due primarily to the main components which were formed in the parts of the nut; those in the kernel being mainly organic, and those in the shuck and shell being for the most part mineral.

The most important stage in the development of the shuck and shell of the pecan was the pre-filling period during which 92.1 per cent. of the dry weight of the shuck and 94.1 per cent. of the dry weight of the shell had been formed, while only 22.4 per cent. of the dry weight of the kernel was evident.

The most critical period in the filling of the kernel was the period prior to September 15, at which time approximately 96 per cent. of the protein, 82 per cent. of the oil, 85 per cent. of the dry weight, and 84 per cent. of the ash of the kernel at maturity date were formed. During the three-week period from August 25 to September 15, 63 per cent. of the total dry weight, 64 per cent. of the total oil, 43 per cent. of the total ash, and 71 per cent. of the total protein were formed in the kernel, which indicated the rapidity of the changes that took place.

The potassium content of the shucks was found to be greatest of the several ash constituents in the pecan fruit, comprising 73 per cent. of all the mineral elements determined in the mature nut, and 34 per cent. of the total weight of the ash of the shuck. Potassium no doubt has a vital function in the translocation or transformation of materials stored in the kernel, since it accumulated at a rapid rate in the shuck and kernel during the early filling stage.

Data show that oil and protein were formed to a large extent in the kernel, while the main mineral constituent found in the shuck was potassium, and calcium in the shell of the pecan.

Magnesium and calcium were found to occur in the smallest amounts in the pecan kernel, while magnesium and phosphorus were lowest in the shuck and shell of the pecan.

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SOME CHEMICAL CHANGES ASSOCIATED WITH THE TRANSITION FROM VEGETATIVE TO REPRODUCTIVE GROWTH OF WINTER WHEAT[1]

G. R. Noggle

(WITH FOURTEEN FIGURES)

The physiology of development of plants has been extensively investigated during recent years. Many different plants have been studied but the results are by no means in agreement. Loehwing (**7**), however, has pointed out that some common relationships seem to characterize certain metabolic processes in typical annuals. Considerable information has been gathered concerning the chemical changes occurring during the transition from the vegetative to the reproductive phase of growth. The vegetative phase of growth of typical annuals is characterized by the rapid intake of water and salts, rapid organic synthesis, maximal water content, and a high respiratory level. During the reproductive phase of growth there is a general trend toward a lower water content and a reduction in the rates of respiration, photosynthesis, and protein formation in the vegetative structures.

It has been possible to exercise some control of the extent and duration of the vegetative and reproductive activities of plants with the discovery of photoperiodism and the conception of "phasic development." The investigator is able to lengthen or shorten the periods of vegetative and reproductive growth of plants by control of temperature and light. Murneek (**8**) made a biochemical investigation of the development of soybeans under short and long photoperiods. The short photoperiod was found to permit sexual reproduction of the soybeans while a long photoperiod kept the plants in the vegetative condition. The short-day plants had a greater percentage increase of dry matter and a higher nitrogen concentration than the long-day plants. The carbohydrate concentration was relatively lower in short- than in long-day soybeans during the first 15–16 days of growth. When young soybean plants were switched from one photoperiod to another, within 9–10 days the concentration of nitrogen and carbohydrate changed so that the resulting concentration was typical of that obtaining under a similar but continuous exposure to the new length of day. The orientation was complete physiologically and morphologically in 17 days. Hibbard (**4**) investigated the changes in activity of catalase, peroxidase, invertase, amylase, and reducase in soybeans under short and long photoperiods. He observed that changes in enzymatic activity could be detected as early as five days after the beginning of the treatments, and that these differences in enzymatic activity could be detected prior to any morphological or chemical changes.

[1] The expenses incurred in the present study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri.

The object of the present study was to determine how soon changes in concentration of certain constituents could be detected in vernalized winter wheat when it was switched from a vegetative photoperiod to a reproductive photoperiod.

Methods

The seeds of a winter wheat (Brill—selection from Turkey Red) were soaked overnight and then placed on clay germinating blocks and held at 2° C. for sixty-seven days. The seeds germinated and the seedlings developed two leaves during the vernalization period. The seedlings were transferred to pots of soil and placed under a daily photoperiod of eight hours. The greenhouse temperature was maintained at 65–70° F. The soil moisture in the pots was maintained at 50 per cent. of the water-holding capacity by weighing the pots on alternate days and adding the water lost by transpiration and evaporation.

Samples were cut at two- to four-day intervals while the plants were on the short-day photoperiod. After the switch-over from short- to long-day, samples were cut daily. All samples were cut at 1:00 P.M. and the green weight of the leaves, stems, and tillers determined. Ascorbic acid and chlorophyll were determined on a composited sample containing fresh leaves, stems, and tillers. Dry weight was determined separately in leaves, stems, and tillers. The samples were then ground in a Wiley mill and stored for chemical analysis.

The ascorbic acid was extracted in a Waring blender with 0.5 per cent. oxalic acid and determined colorimetrically by the method of LOEFFLER and PONTING (**6**). Chlorophyll was determined by the method of COMAR, BENNE, and BUTEYN (**1**). Riboflavin was assayed microbiologically using *Lactobacillus casei*. The micro-Kjeldahl procedure was used to determine the total nitrogen content.

Data and discussion

The eight-hour photoperiod was not adequate to permit the plants to enter the reproductive phase of development. The growing point remained undifferentiated and new leaves were formed until the plant had nine leaves. The plants were then transferred to a daily photoperiod of fourteen hours. Within ten days the plants showed evidences of jointing and the growing point differentiated floral primordia.

During the course of the experiment, measurements of the height of the plants were taken at weekly intervals (fig. 1). Several periods of change in growth rate were observed following the switch-over from short- to long-days. These changes in growth rate are especially evident when the weekly increments of increase in height are plotted as shown by figure 2. There was a considerable increase in height immediately following the switch-over. During the following week there was little increase in height of the plants. The period probably was one of considerable internal readjustment both morphologically and physiologically. Another period of increased growth

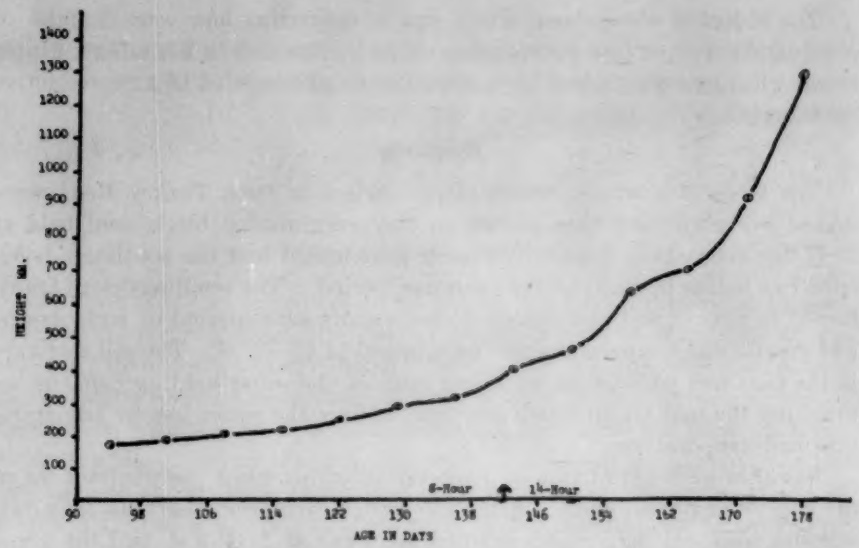

FIG. 1. Total height of plants at weekly intervals.

was evident during the following week. The increase in height was associated with the elongation of the internodes and lengthening of the stem. The next week was characterized by a decreased growth increment. During this week the boot leaf developed and the spike formed. There was but little

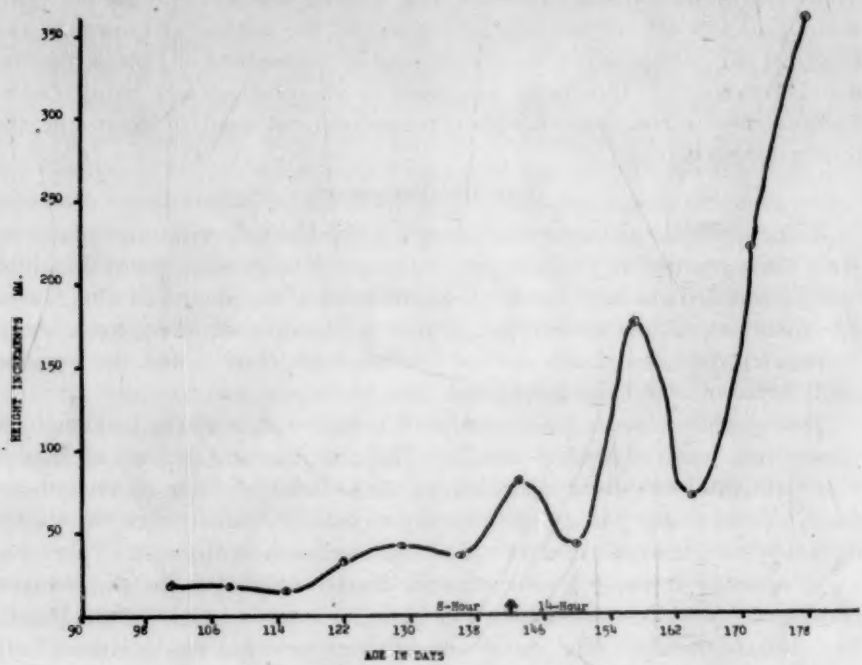

FIG. 2. Weekly increments of height.

increase in height due to stem elongation. The plants steadily increased in height due to the emergence of the spike and the elongation of the grain stalk during the following weeks.

The total green weights of the wheat plants are shown in figure 3. There was a drop in the total green weight two days after the plants were switched from short- to long-days. During subsequent samplings, there were several other fluctuations in green weight, but the general trend remained toward an increased green weight. Figure 4 shows the green weight of leaves and stems. The leaves showed considerable decrease in green weight after the switch-over. The stem appeared after the plants were exposed for several

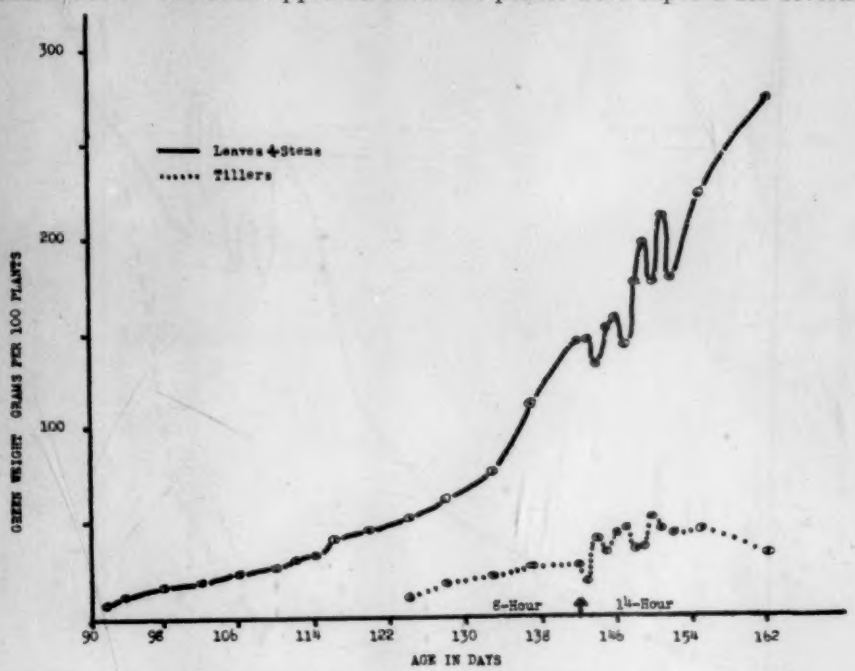

FIG. 3. Green weights of plants (leaf and stem) and of tillers.

days to the long photoperiod and soon dominated the total green weight of the plant.

The total dry weight, as seen in figure 5, showed some irregularities following the switch-over from short- to long-days. From the data presented in figure 6, it is seen that there was an immediate decrease in dry weight of leaves after the switch-over. The dry weight of the leaves recovered and then increased. The stems showed two periods of slow increase in dry weight followed by a period of rapid dry weight production.

The initial decrease in green weight production following the switch-over was probably due in part to a decreased moisture content. An examination of the growing point during this period indicated that flower primordia were being differentiated. LOEHWING (7) has pointed out that a loss of tissue

moisture is characteristic in annuals at the time of initiation of flower buds. However, some of the wide irregularities in green weight might be due to sampling differences. The decrease in dry weight production of the leaves was associated with the increase in dry weight of the stems. It is likely that translocation of various constituents took place from leaves to stems and accounted for the decrease in dry weight of the leaves and increase of the stems.

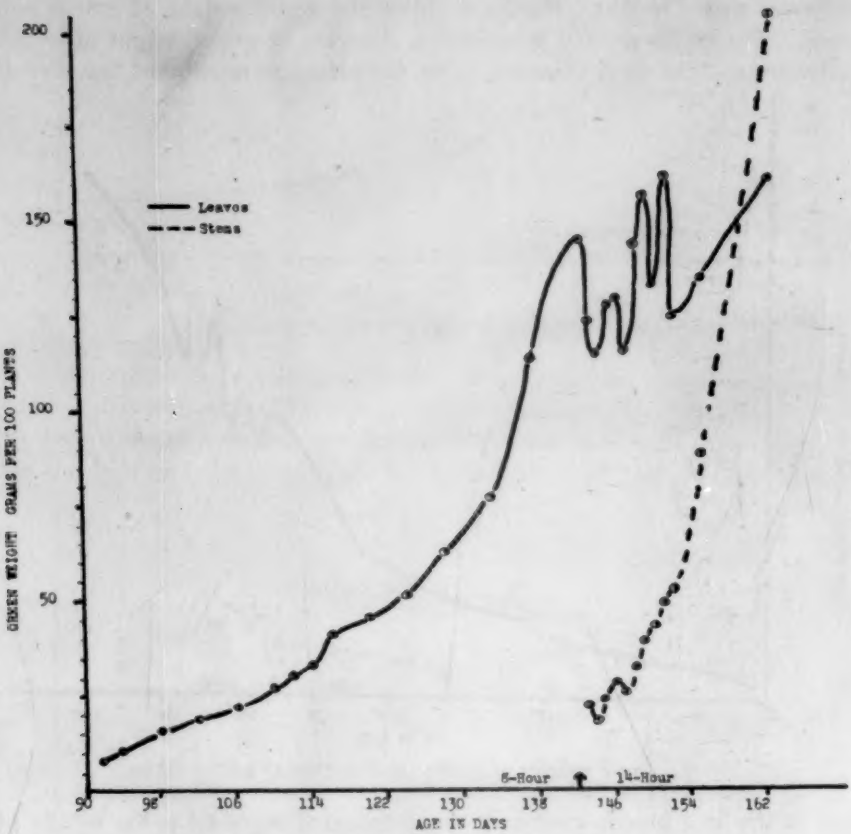

FIG. 4. Green weights of leaves and stems.

The weekly increments of green and dry weight production of the total plant are shown in figure 7. During the period immediately following the switch-over the green weight production was at a standstill. This period of decreased production was followed by two periods of accelerated green weight production. The increments of dry weight do not indicate any such decreased production as was noted in the green weight curve. This situation suggests that the moisture in the tissue was the constituent most seriously affected. Some deviations in the dry weight increment curve might have been found if different sampling dates had been used.

WITTWER (11) demonstrated that several periods of accelerated growth

of corn were associated with synapsis and syngamy. He found that increased growth hormone production during these periods was responsible for the accelerated rates of growth. In the present experiment, synapsis was observed but syngamy did not occur. The fluctuations in the growth are likely associated with some phase of the synaptic process but the nature of the relationship is not apparent.

A high concentration of total nitrogen was maintained in the young wheat plant as shown by figure 8. The nitrogen concentration dropped off

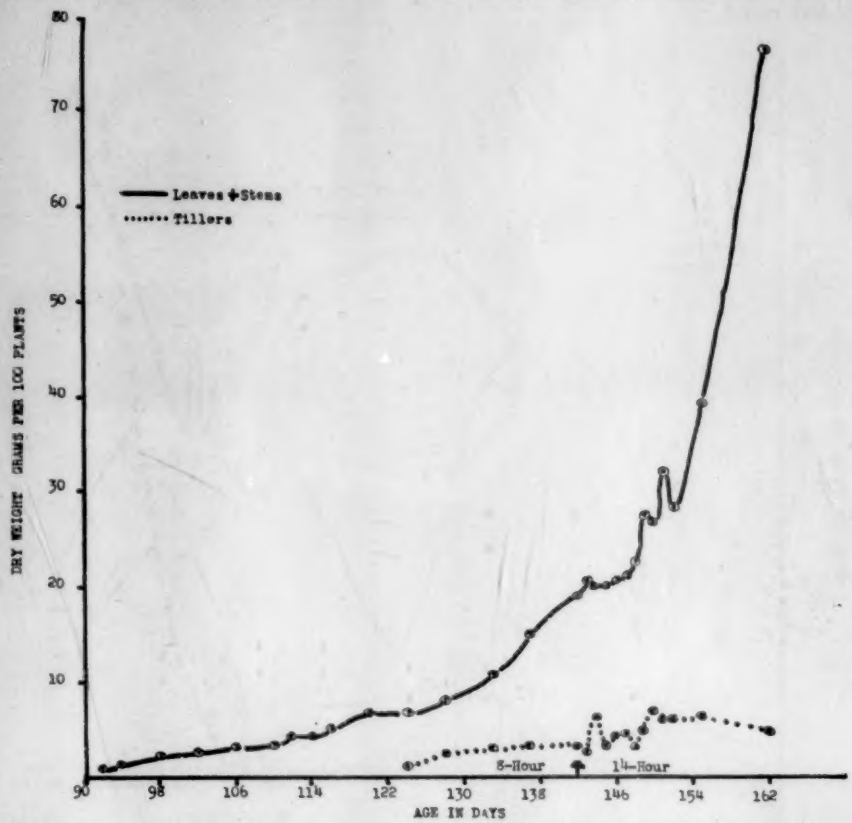

FIG. 5. Dry weights of plants (leaf and stem) and of tillers.

in the later vegetative stages and following the switch-over from short- to long-days the per cent. nitrogen rapidly decreased. The regular fluctuations in the percentage of nitrogen which followed the switch-over are difficult to explain. The dry weight of the plant did not show such fluctuations, but the points of maximum nitrogen concentration occurred on days when the leaves had a high green weight or moisture content.

The green weight of the stems did not show fluctuations similar to those noted in the leaves. The nitrogen content, however, of the stems followed that of the leaves. This would indicate that the nitrogen was being synthe-

sized in the leaves and rapidly translocated to the stems. The parallel fluctuations of nitrogen in leaf and stem suggest that the altered photoperiod has induced internal metabolic changes, the nature of which are unknown. The green and dry weights of the tillers showed little similarity to the green and dry weights of the leaf or stem tissue but the fluctuations in nitrogen content of the tillers followed closely those noted for leaf and stem. The similarity of nitrogen changes in leaf, stem, and tiller suggests that the nitrogen is related to some internal metabolic disturbance set off by the change in photoperiod.

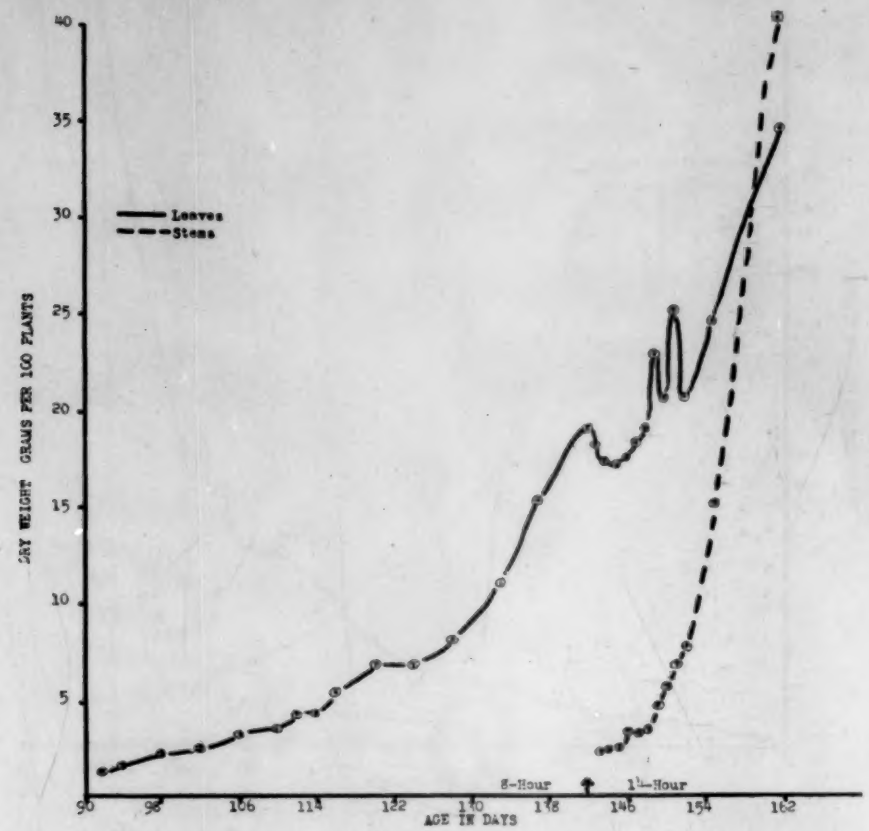

FIG. 6. Dry weights of leaves and stems.

The riboflavin concentration of the plant tissue is shown in figure 9. The riboflavin showed an early maximum concentration and then diminished steadily as the plant matured. Following the switch-over from short- to long-day photoperiod the concentrations of riboflavin varied similarly to the total nitrogen concentrations. There was one difference, however, in that immediately following the switch-over the nitrogen content sharply dropped and then recovered on the 145th, 148th, and 151st days while the riboflavin concentration did not decrease following the switch-over but sharply in-

creased in concentration on the 145th, 148th, and 151st days. Leaves, stems, and tillers showed trends in riboflavin concentration identical with those noted for the nitrogen content of the leaves, stems, and tillers. The similarity of behavior of the nitrogen and riboflavin suggests that the two constituents are related to some internal metabolic changes brought about by the altered photoperiod.

Figure 10 shows that the ascorbic acid concentration of the fresh tissue fluctuated widely during the growing period of the plant. The trend was

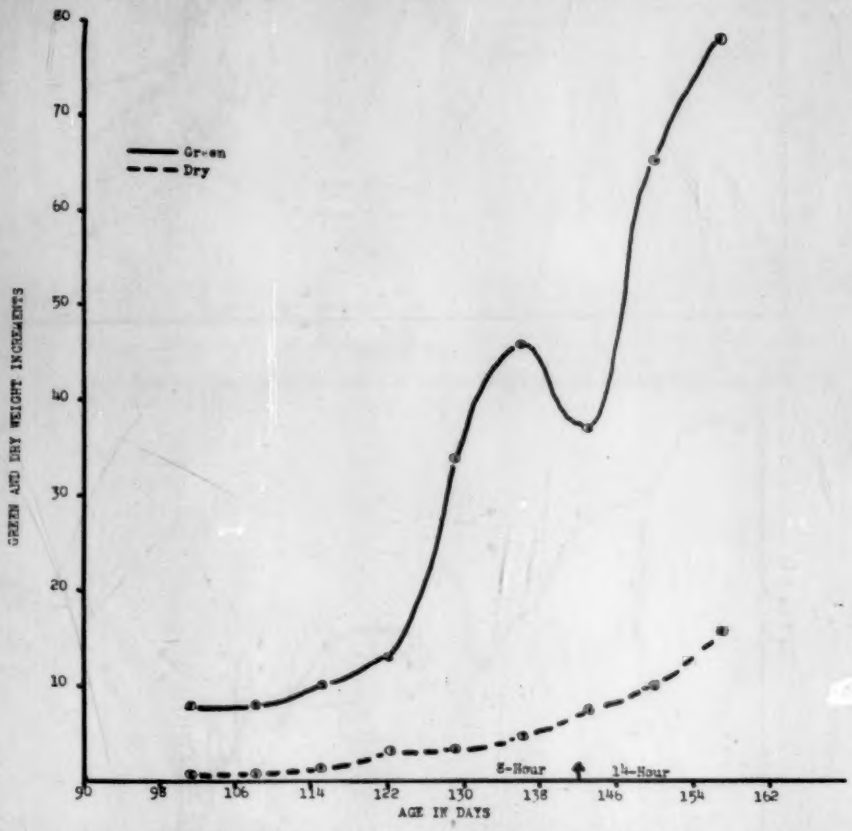

FIG. 7. Weekly increments of green and dry weight.

toward a decreased concentration of ascorbic acid as the plant matured. Following the switch-over, the ascorbic acid content attained three peaks in concentration on the 144th, 147th, and 150th days. These peaks in ascorbic acid concentration coincided with days on which the green weight of the plant was at a minimum. It was pointed out that the fluctuations in green weight were mainly due to fluctuations in the moisture content of the tissue. It thus appears that the ascorbic acid concentration is dependent upon the degree of hydration of the plant tissue. That the degree of tissue hydration is not entirely the cause of the fluctuations in ascorbic acid concentration

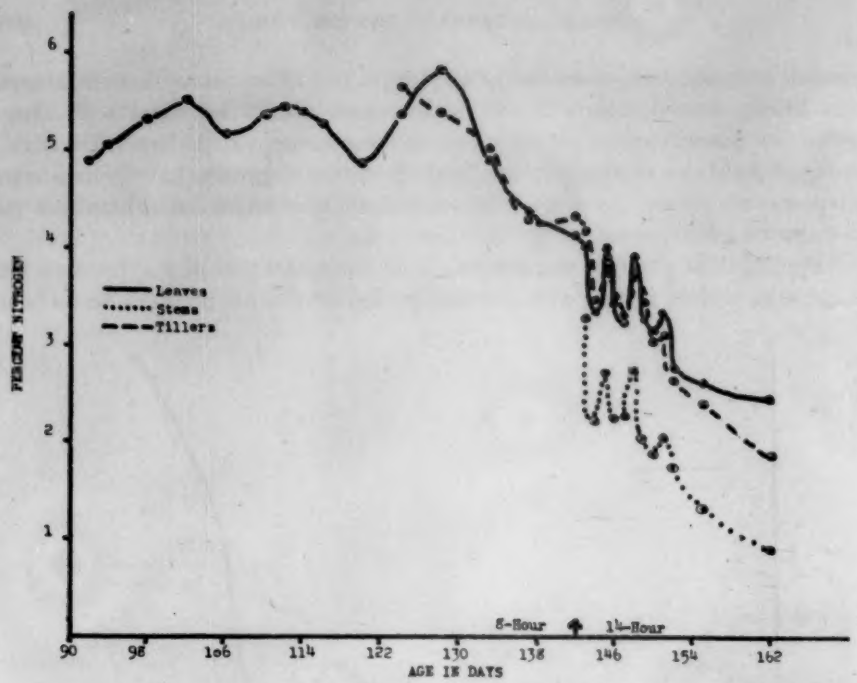

FIG. 8. Percentage of nitrogen in dry samples of leaves, stems and tillers.

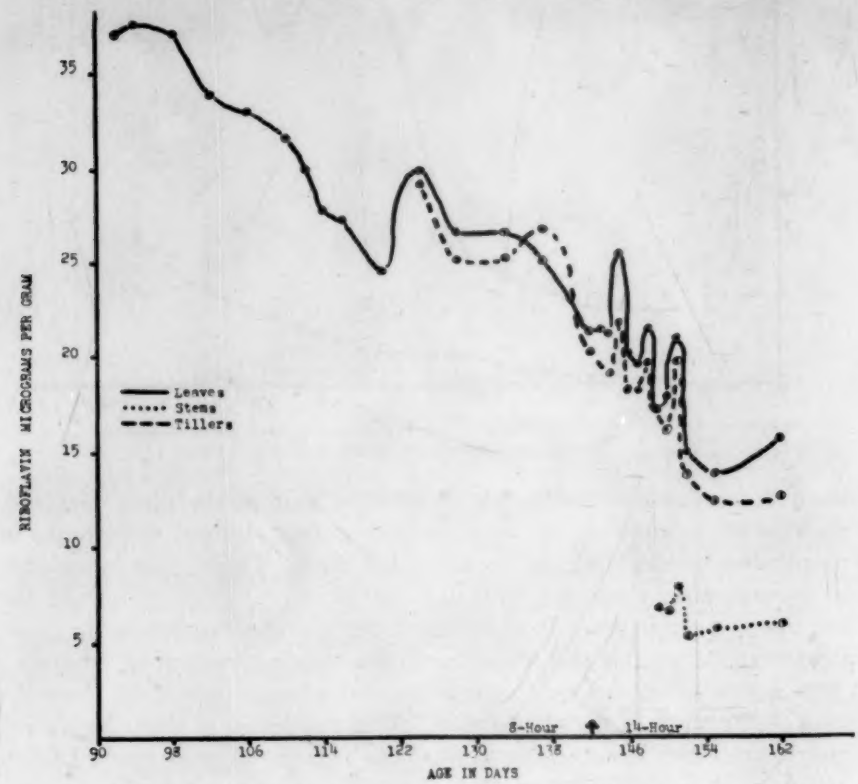

FIG. 9. Micrograms of riboflavin per gram of dry tissue of leaves, stems, and tillers.

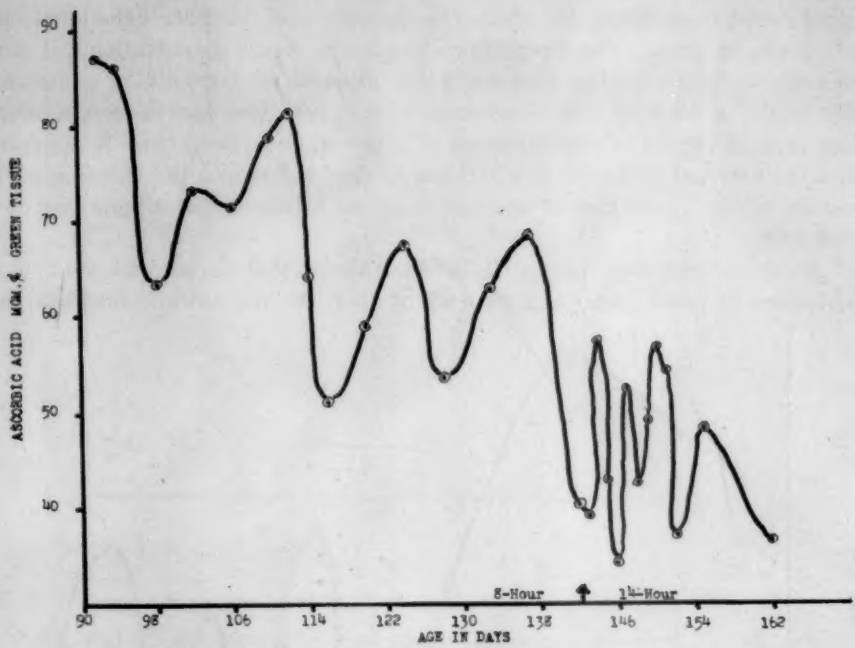

FIG. 10. Milligrams of ascorbic acid per 100 grams of fresh plant tissue.

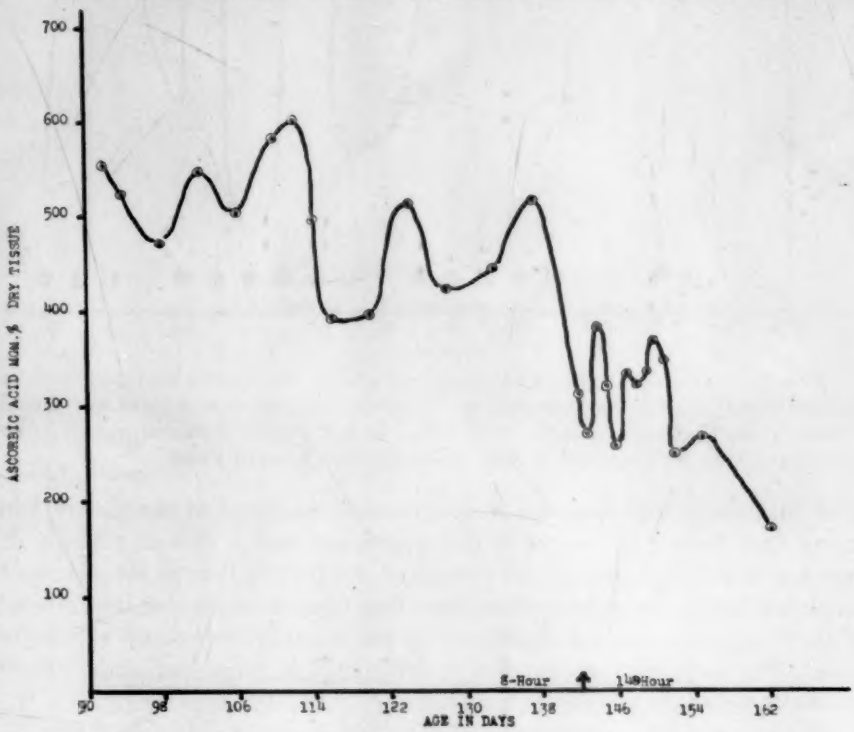

FIG. 11. Milligrams of ascorbic acid per 100 grams of dry plant tissue.

can be seen from figure 11, where the ascorbic acid has been calculated on a dry weight basis. The fluctuations in ascorbic acid concentration did not coincide with the fluctuations noted for nitrogen and riboflavin contents. The peaks in ascorbic acid concentration occurred one day earlier in each case than the peaks in concentration of nitrogen and riboflavin. It appears that the internal processes in plant metabolism influenced the ascorbic acid concentration in a different manner than the nitrogen and riboflavin concentration.

A number of investigations (**3, 10**) have shown that the ascorbic acid concentration of plant material is dependent upon the amount and duration of

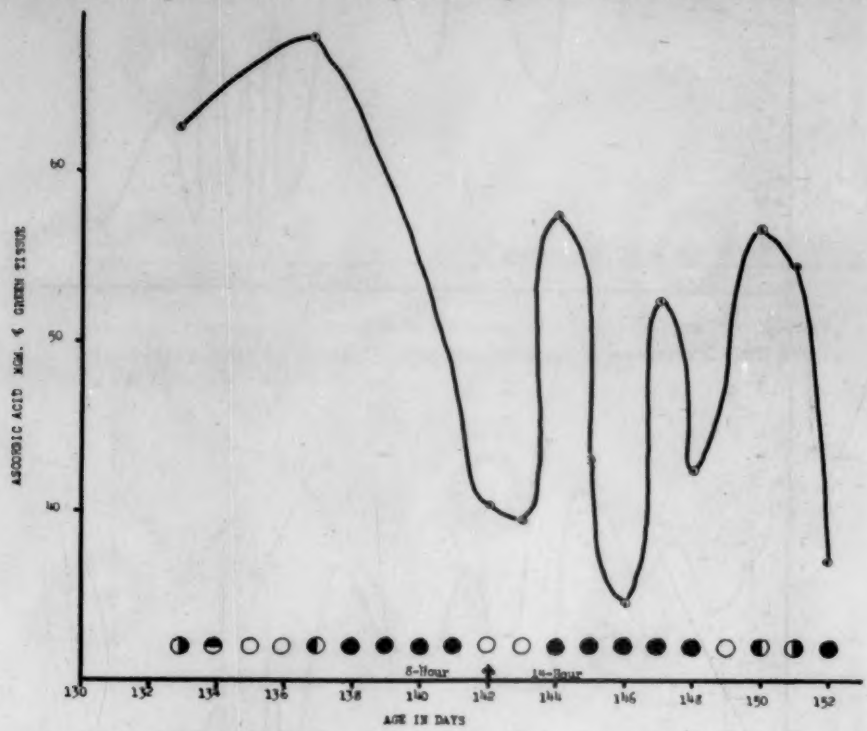

FIG. 12. Influence of daily amounts of sunlight on the ascorbic acid concentration of plant tissue. ○ = Sunlight all day; ● = cloudy all day; circle with right half shaded = morning clear, afternoon cloudy; circle with left half shaded = morning cloudy, afternoon clear; circle with top half shaded = intermittently clear and cloudy.

light that the plants receive. A daily record was kept of the cloudy and sunny days during the course of the experiment and is shown in figure 12. One might possibly account for several of the fluctuations in ascorbic acid concentration by the amount of sunlight that the plants received, but several of the fluctuations were independent of the durations of sunny or cloudy days. The data suggest that the ascorbic acid is connected with certain phases of metabolic activity in the plants.

The chlorophyll concentration of the fresh tissue is shown in figure 13.

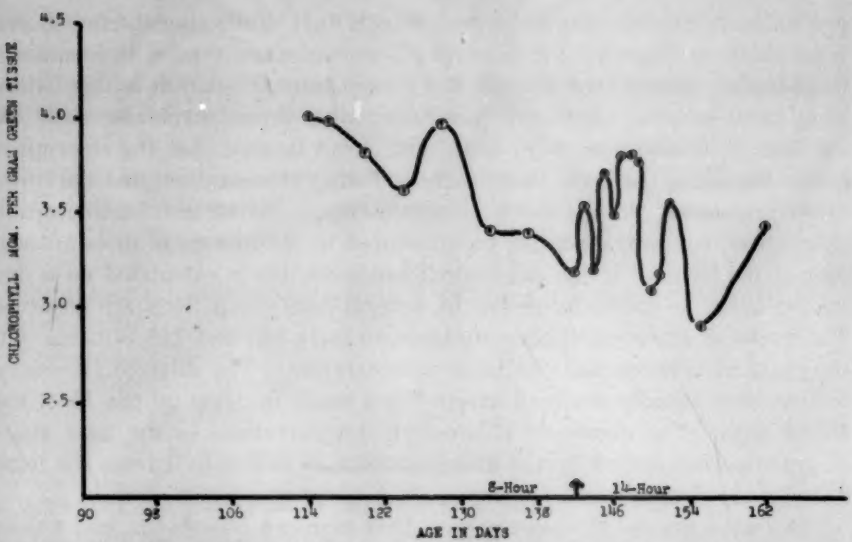

FIG. 13. Milligrams of chlorophyll per gram of fresh plant tissue.

With the exception of the high chlorophyll concentration on the 128th day, the chlorophyll decreased as the plant matured. No explanation is suggested for the high chlorophyll concentration on the 128th day, but the nitro-

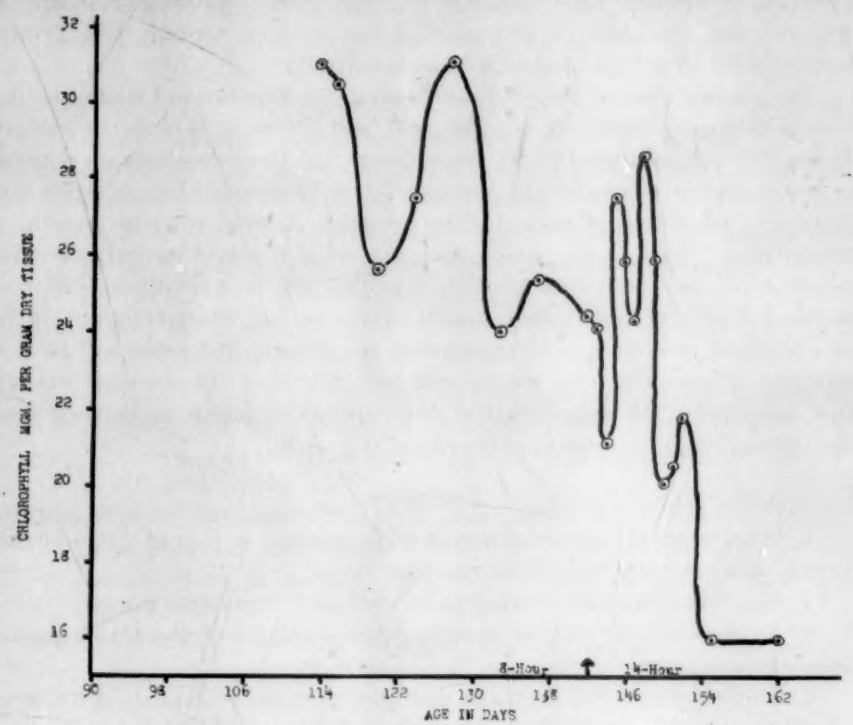

FIG. 14. Milligrams of chlorophyll per gram of dry plant tissue.

gen concentration also showed a peak on this day. Following the switch-over from short- to long-day, the chlorophyll concentration tended to increase in magnitude. Several workers (**2, 8, 9**) have pointed out that a high chlorophyll and carotene content is associated with reproductive activity. No carotene determinations were made, but it can be seen that the chlorophyll concentration of the plant tissue increased when the plant entered the reproductive phase of development. Undoubtedly, some of the fluctuations in chlorophyll concentration can be attributed to differences in moisture content of the tissue. If the chlorophyll concentration is calculated on a dry-weight basis, as shown by figure 14, several interesting facts are observed. The peaks of chlorophyll concentration on days 145 and 148 coincide with the peaks in nitrogen and riboflavin concentration. The chlorophyll concentration then rapidly declined except for a small increase on the 151st and 152nd days. The decreased chlorophyll concentrations in the later stages of growth were caused by the disappearance of chlorophyll from the lower dead leaves of the plants.

The data presented above suggest that nitrogen, riboflavin, and chlorophyll behave similarly during the transition from vegetative to reproductive growth. The ascorbic acid does not seem to be directly related to these constituents. Nitrogen and riboflavin are known to be high in tissues with a high respiratory activity (**5, 7**). Chlorophyll and carotene have been suggested to be associated with the reproductive phase of growth (**2, 8, 9**). It appears that the changed photoperiod has induced reproductive growth accompanied by a high level of metabolic activity.

The present state of knowledge regarding the functions of such constituents as nitrogen, riboflavin, ascorbic acid, and chlorophyll is rather meager. It has been demonstrated in this experiment that these constituents changed in concentration when the plant was switched from conditions of vegetative growth to conditions of reproductive growth. Altered rates of growth, as measured by total height, green and dry weight, also occurred when the plants were switched from vegetative to reproductive conditions. A more detailed determination of such constituents as carbohydrate fractions, nitrogen fractions, minerals, enzyme systems, and growth hormones will have to be made before a complete picture can be obtained of the chemical changes that accompany the morphological development of plants as they go from the vegetative to the reproductive phase of growth.

Summary

1. Winter wheat was vernalized at 2° C., planted in pots of soil, and then grown under a daily eight-hour photoperiod.

2. The short-day photoperiod permitted only vegetative growth.

3. Following a switch-over to a long-day photoperiod, the plants jointed and produced grain.

4. There were several rhythmic fluctuations in concentration of nitrogen, riboflavin, ascorbic acid, and chlorophyll following the switch-over from short- to long-day conditions.

5. The nitrogen, riboflavin, and chlorophyll behaved similarly following the switch-over.

6. The fluctuations in ascorbic acid concentration occurred a day earlier than the fluctuations in concentration of nitrogen, riboflavin, and chlorophyll.

7. The changes in concentration of nitrogen, riboflavin, ascorbic acid and chlorophyll were detected before external evidences of flowering were evident.

The author is indebted to Dr. F. L. WYND for his advice during the course of this research.

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RESPIRATION OF COTTONSEED

M. L. KARON AND A. M. ALTSCHUL

(WITH FIVE FIGURES)

Introduction

MALOWAN (**12, 13**), in an investigation of the chemical and physical changes that take place in cottonseed during storage, observed that when the moisture content of prime cottonseed was artificially raised, its rate of respiration was also increased. When moist seeds which had a high rate of respiration were put in thermally insulated containers, a rise in the temperature of the seeds was noted. The highest temperature observed under Malowan's experimental conditions was 157° F., although he had noted temperatures up to 175° in carloads of seeds received at oil mills. BAILEY and coworkers (**3, 4, 5**) have extensively studied the respiration and storage behavior of cereal grains. RAMSTAD and GEDDES (**15**) investigated the behavior of soybeans in this respect. LARMOUR *et al.* (**10**) investigated the respiration of sunflower seed and flaxseed.

These and other investigations revealed several generalizations which may be recapitulated as follows:

1. Under carefully controlled experimental conditions, the respiration intensity of seeds, as measured by their rate of carbon dioxide production, is a regular function of their moisture content. The curves obtained by plotting respiration intensity against the moisture content of the seeds are logarithmic in character. This relationship between moisture content and respiration intensity holds, however, only when all the experiments are made on samples of the same lot of seeds and when each respiration measurement is made exactly the same length of time after the seeds are conditioned.

2. Broken and shriveled seeds, kernels, and flakes have higher respiration intensities than clean, intact seeds of the same type and moisture content.

3. The rate of respiration of seeds varies with respect to a number of factors, such as length of exposure to moisture, age, variety, conditions of growth, and other indeterminate properties. It is not possible, then, on the basis of moisture content alone, to predict respiration intensity. Similarly, it has been previously shown (**8**) that it is impossible to predict the rate of free fatty acid production in cottonseed on the basis of moisture content alone.

4. Oilseeds respire at a higher rate than do cereal grains of the same moisture content.

The purpose of the present investigation has been to determine the effect of such variables as moisture content, variety, location of growth, and age on the respiration of cottonseed. For convenience in expressing the effect of these variables in a quantitative manner, a quantity designated "average respiration intensity" (RI) has been proposed, and a means for its measurement has been developed.

Description of the cottonseed samples

The samples used in these experiments were of three different varieties of cottonseed grown at three widely separated Agricultural Experiment Stations. Further diversity was introduced by the circumstance that samples of the same variety were taken at different times during the cotton-harvesting season. Thus, a wide cross section of cottonseed, differing as to variety, locality, and date of harvesting, was available for investigation.

The seed samples used in the respiration experiments are described in table I. Those designated as 104, 204, and 304 were from bolls which had

TABLE I

DESCRIPTION OF SAMPLES OF *Gossypium hirsutum* USED IN THE RESPIRATION INVESTIGATIONS

SAMPLE	VARIETY	DATE PLANTED	DATE OF HARVESTING	FREE FATTY ACID CONTENT	MOISTURE* CONTENT
				%	%
104	Delfos 3506†	.....	Aug. 29, 1942	1.48	12.0
105	"	.....	Oct. 3, 1942	0.90	10.6
204	Coker's 200, strain 1‡	April 20, 1942	Sept. 22, 1942	7.12	13.7
205	"	"	"	0.99	10.2
206	"	"	Oct. 22, 1942	0.76	10.0
304	Oklahoma Triumph, 0-52-12§	.....	Sept. 22, 1942	1.62	10.5
305	"	.....	Oct. 1, 1942	1.04	10.1
306	"	.....	Oct. 28, 1942	1.04	10.6
307	"	.....	Nov. 28, 1942	0.85	9.3
308	"	.....	Dec. 28, 1942	0.81	15.7

* This refers to the percentage of moisture of the seeds as received, on a wet basis.
† Grown at Stoneville, Mississippi.
‡ Grown at Clemson, South Carolina.
§ Grown at Stillwater, Oklahoma.

just begun to crack open, being, therefore, typical seeds from an early or premature harvest of cotton. The next series of samples (105, 205, and 305) was taken from a normal harvest of cotton and the remaining samples were from cotton that had been kept in the field from one to three months after maturation before being harvested.

It will be observed that natural variations existed in the moisture contents of the seed samples as received in the laboratory. In addition, portions of all samples were treated so as to artificially change the moisture content to values above or below the natural condition. It was thus possible to measure the effect of moisture on seed properties over a wide range of natural and artificially induced moisture content.

Experimental method

Three-pound lots of seed were set aside for each series of measurements. Whenever it was necessary to change the moisture content artificially either

above or below its original value, care was taken to so condition the seeds to the desired moisture content that any resulting biochemical activity would be minimized and permanent injury to the seeds would be prevented. Conditioning was accomplished in this manner by carrying out all moisture changes in a cold room, as previously described (**8**). Each 3-pound lot of seeds was then stored in air-tight jars at 25° C.

In order to measure the respiration of the seeds in any one lot, a 10-, 25-, or 50-gram sample was withdrawn from the storage container and placed in a stoppered respiration flask (**1**) for a length of time considered sufficient to produce a significant change in the atmosphere in the flask. A sample of gas, withdrawn from the flask, was then analyzed for oxygen and carbon dioxide by means of the apparatus and procedure described in a preceding publication (**1**). Upon completion of the analysis, the seed sample in the respiration flask was discarded, and fresh samples were used for subsequent respiration measurements, the size of the sample and the length of the respiration interval being based on the results of the previous analysis. Thus, for example, when measurements indicated a rising rate of respiration for a lot of seeds, the size of the subsequent samples and the length of the respiration testing intervals were reduced accordingly.

Respiratory quotient

Both oxygen and carbon dioxide were measured in each respiration analysis, thus making it possible to calculate the ratio of carbon dioxide evolved to oxygen absorbed, or the respiratory quotient (RQ), in resting cottonseed. Complete respiration patterns were obtained on 17 different

TABLE II

THE VARIATION OF THE RESPIRATORY QUOTIENT (RQ) OF COTTONSEED DURING STORAGE

SAMPLE	105e		205e		307a	
MOISTURE CONTENT	16.8%		15.9%		12.9%	
	LENGTH OF STORAGE	RQ	LENGTH OF STORAGE	RQ	LENGTH OF STORAGE	RQ
	days		*days*		*days*	
	15	0.96	46	0.92	15	0.91
	49	1.08	91	0.93	51	0.94
	79	0.99	116	0.99	86	0.97
	130	0.98	144	1.05	162	0.96
	146	1.00	.....	.....	.....	.....
	167	0.94	.....	.....	.....	.....
	192	1.12	.....	.....	.....	.....
	239	0.85	.....	.....	.....	.....
AVERAGE RQ	.....	0.99	.....	0.97	.....	0.95

lots of cottonseed, in none of which was there any regular progression of the value of the RQ during the course of the storage period. Instead, all the fluctuations observed were of a random nature, as illustrated by the ex-

amples given in table II. The values of the RQ for each lot of seeds were, therefore, averaged over the complete storage period, with the results shown in table III.

It is quite evident from an examination of these results that the value for the respiratory quotient of resting cottonseed is very nearly unity and is independent of moisture content, variety, place of growth, and harvesting period. Seeds with artificially adjusted moisture contents gave results identical with those allowed to retain their natural moisture contents.

Inasmuch as the RQ of resting cottonseed was found to be unity, it thus becomes possible to determine its respiration by means of a single measurement of either the carbon dioxide evolved or the oxygen absorbed. The

TABLE III

THE AVERAGE RESPIRATORY QUOTIENT (RQ) OF STORED COTTONSEED SAMPLES

SAMPLE	MOISTURE CONTENT	RESPIRATORY QUOTIENT	GROUP AVERAGE
	%		
104b	10.0	0.95	
105b	10.7	1.02	
104a	12.0	1.01	
105a	13.1	1.05	
105f'	14.9	1.00	
105e	16.8	0.99	1.00
204b	12.5	0.95	
205a	12.5	1.04	
204a	13.7	0.98	
206a	14.7	0.93	
205e	15.9	0.97	0.98
305b	10.1	1.01	
306b	10.6	0.96	
307a	12.9	0.94	
306a	13.1	1.02	
304a	13.3	1.09	
308a	15.7	0.95	1.00
Average respiratory quotient			0.99

respiration data in this publication, therefore, are presented in terms of carbon dioxide evolution, it being understood that the amount of oxygen absorbed is equivalent to the amount of carbon dioxide evolved.

Simply interpreted, a respiratory quotient of unity indicates that carbohydrate is being metabolized in the respiration process. Cottonseed kernels contain approximately 14 per cent. carbohydrates, of which 8 per cent. is soluble carbohydrates (**14**). Thus, there is sufficient carbohydrate in the seeds to sustain the respiration that takes place during the storage period. MALOWAN (**13**) has actually demonstrated that there is a measurable decrease in carbohydrate content of cottonseed after the seeds have heated in storage. This observation is paralleled in work by RAMSTAD and GEDDES (**15**), who, on the basis of their calculations of the specific heat of resting soybeans, concluded that carbohydrates are also the first materials utilized in soybean respiration.

Pattern of respiration

The results of respiration measurements on the samples of seeds described in tables I and III are plotted in figures 1, 2, and 3. In these figures the curves are the best smooth curves that could be drawn from the experimental data. Each bar represents a respiration measurement; the height of the bar above the horizontal axis is equal to the average rate of respiration for the period during which the seeds were confined in the respiration flask, and

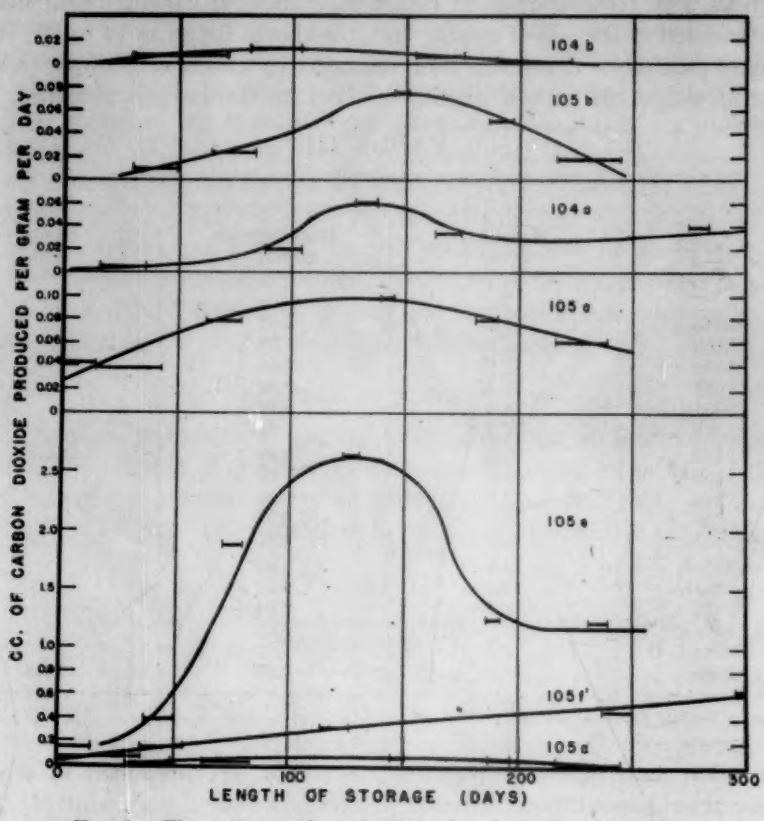

Fig. 1. The patterns of respiration of seeds in the 100 series.

the length of the bar is equal to the test interval. In order to present on one graph the respiration curves for each sample in a series, the ordinate scale in each case has been adjusted to the intensity of respiration of the sample.

Most of the curves in the above-mentioned figures exhibit a maximum rate of respiration during the storage period under investigation. It is of interest that this maximum rate is roughly proportional to moisture content. For example, in the 100 series, an increase in moisture content from 10 to 13.3 per cent. is paralleled by an increase in the maximum from 0.014 to 0.105 cc. of carbon dioxide per gram per day. At the 16.8 per cent. moisture

level, the maximum rate of respiration is 2.64 cc. of carbon dioxide per gram per day.

Intensity of respiration

One difficulty encountered in the correlation and interpretation of the respiration data of different investigators arises from the use of an almost instantaneous respiration rate as a measure of the intensity of respiration of the seeds. Thus respiration intensity has been generally defined as the rate of production of carbon dioxide by a fixed weight of seeds kept for a stated interval from the time received at the laboratory or from the time

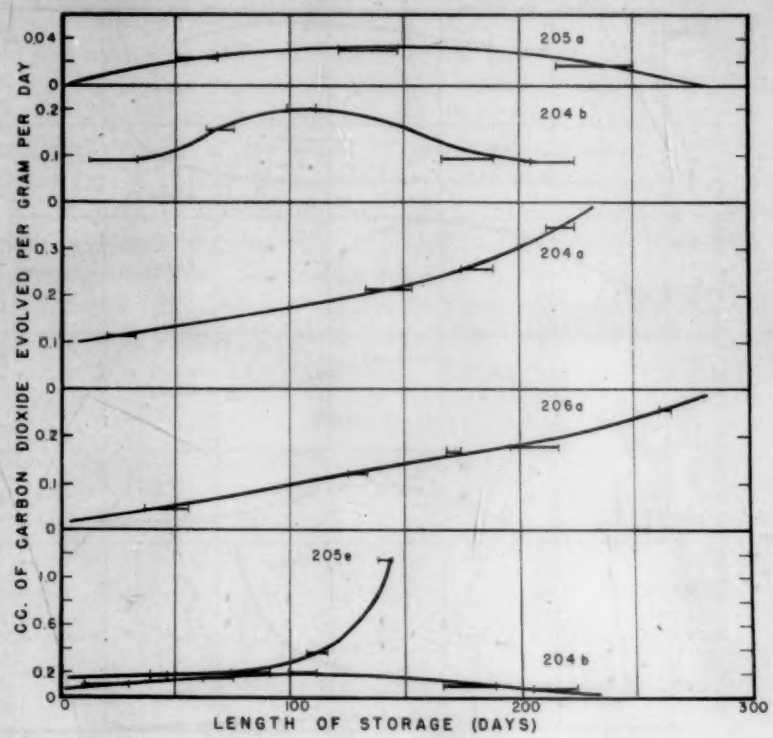

Fig. 2. The patterns of respiration of seeds in the 200 series.

conditioned to the desired moisture content. This interval may vary from a few days to years, depending on the convenience and objectives of the investigator. But such a limited definition of intensity is inadequate to provide an overall representation from the time the seeds are received until they are milled or planted, since the age and conditions of storage have a profound and often unpredictable effect on the instantaneous rate of respiration. For example, the rate of respiration measured five days after the seeds are received may have an entirely different intensity from that obtained when the rate of respiration is measured on the same seeds 30 days later. There is no *a priori* reason to prefer one measurement to the other,

and in neither case can the measured rate of respiration be properly termed the respiration intensity of the seeds.

The confusion that might be occasioned by making instantaneous rate measurements at different intervals during the storage period is illustrated by the following cottonseed respiration data: After 50 days of storage, sample 105e respired at a rate which was approximately three times as great as that of 105f′ and eight times as great as that of 105a (fig. 1). After 100 days' storage, the ratio of respiration rates of the same samples was approximately 36:2.9:1. This inconsistency is even more strikingly demon-

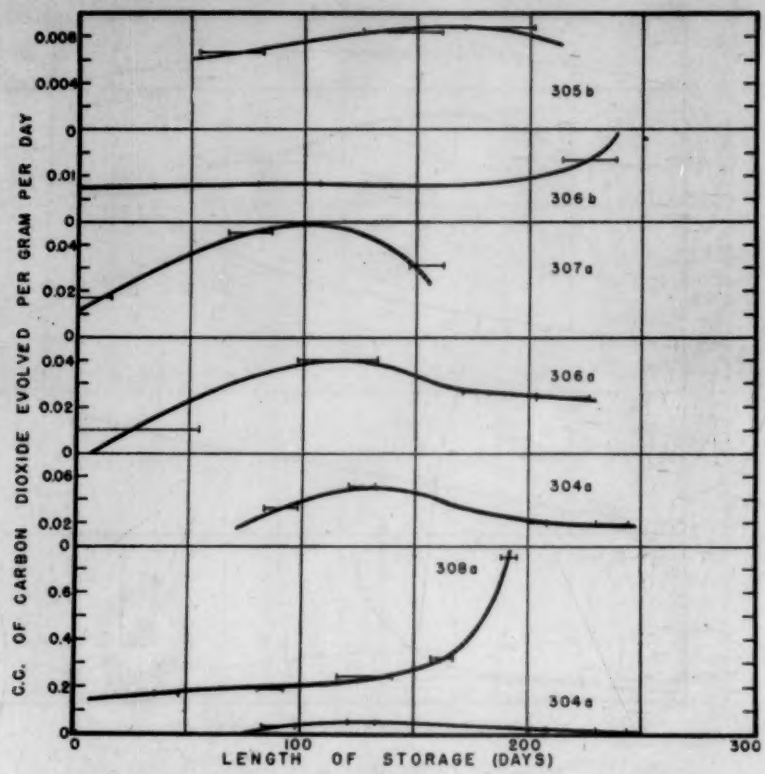

FIG. 3. The patterns of respiration of seeds in the 300 series.

strated in a comparison of the respiration rates of samples 205e and 204b (fig. 2). For the first 75 days' storage both samples had practically the same rate of respiration. It was only after this period that the curves began to diverge sharply.

Any attempt to formulate a definition of respiration must be based on the knowledge that I, the instantaneous respiration rate, is an unknown function of the time, t. For the purposes of this investigation, therefore, an averaging procedure was used and a quantity termed "average respiration intensity," has been defined by the following equation:

$$(RI) = \frac{\int_{t_o}^{t_f} I dt}{t_f - t_o} \qquad (1)$$

where (RI) = average respiration intensity,
I = instantaneous respiration rate at time, t,
t_o = beginning of experiment,
t_f = end of experiment, and
$t_f - t_o$ = duration of experiment.

In order to evaluate (RI), periodic measurements of respiration rate were made on samples of the same seed lot over a long interval of time. All of the samples were observed over periods of not less than 100 days and generally over 200 days. The measurements so obtained formed a respiratory pattern for a given sample of cottonseed. Thus, upon integration of these patterns and division of the result by the total number of days elapsed during the experiment, an average daily respiration rate (RI) was obtained for each sample. In spite of its empirical nature, this definition, because it covers a longer period, may approach more closely than previous representations a "true" respiration intensity of resting seeds.

The value of (RI), average respiration intensity, was calculated for all of the samples illustrated in figures 1, 2, and 3, by first measuring the area under the curves by use of a planimeter. Extrapolations were avoided by

TABLE IV

VALUES FOR MAXIMUM AND AVERAGE RESPIRATION RATES OF COTTONSEED SAMPLES

SAMPLE	MOISTURE CONTENT	MOISTURE TREATMENT*	TOTAL RESPIRATION	STORAGE INTERVAL	AVERAGE RESPIRATION INTENSITY (RI)	MAXIMUM RATE OF RESPIRATION
	%		*cc. CO_2 per gram*	*days*	*cc. CO_2 per gram per day*	*cc. CO_2 per gram per day*
104b	10.0	Lowered	1.58	193	0.0082	0.014
105b	10.6	None	9.46	216	0.044	0.076
104a	12.0	None	8.16	269	0.030	0.062
105a	13.1	Raised	18.10	239	0.076	0.105
105f'	14.9	Raised	110.8	300	0.37	.....
105e	16.8	Raised	362.6	239	1.42	2.64
205a	12.5	Raised	5.44	202	0.027	0.023
204b	12.5	Lowered	29.45	212	0.14	0.20
204a	13.7	None	42.3	204	0.21	.....
206a	14.7	Raised	32.8	230	0.14	.....
205e	15.9	Raised	38.2	105	0.36	.....
305b	10.6	None	1.19	148	0.0080	0.0087
306b	10.6	None	2.09	238	0.0088	.....
307a	12.9	Raised	5.88	162	0.036	0.049
306a	13.1	Raised	6.05	226	0.027	0.040
304a	13.3	Raised	5.34	161	0.033	0.051
308a	15.7	None	44.6	158	0.282	.....

* Refers to any conditioning treatment which may have been applied initially to either raise or lower the moisture content of the seed sample before it was set aside for respiration measurements.

measuring only that portion of the area that was in the interval between the beginning of the first and the end of the last experimental bar in each respiration pattern. By dividing the volume of carbon dioxide produced by the number of days elapsed during the experimental interval, the value of (RI) was obtained.

The results of these calculations are given in table IV together with values for the maximum respiration rates of those samples which exhibited maxima and a description of the moisture conditioning treatment employed before the seed samples were set aside for the respiration studies.

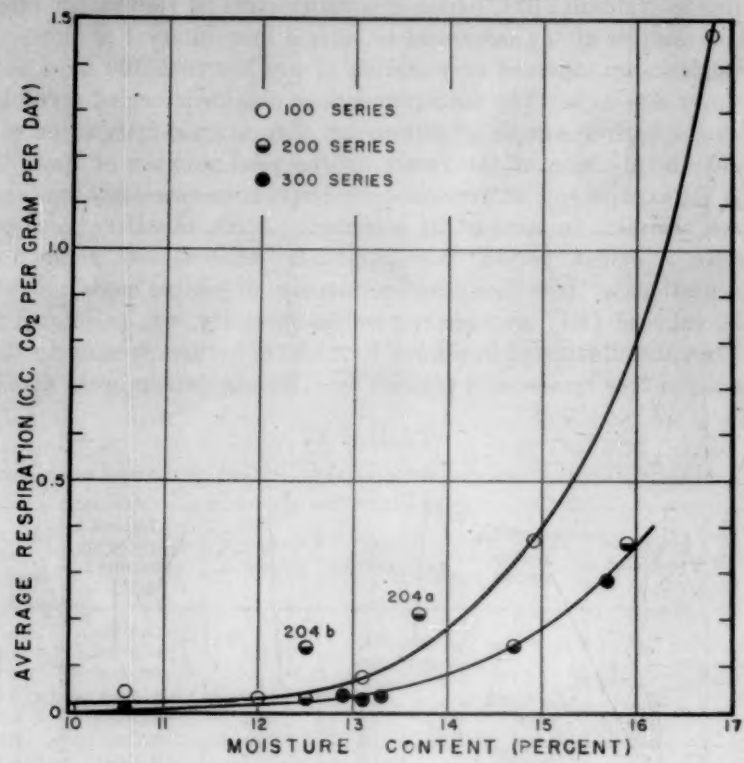

FIG. 4. The effect of moisture content on the intensity of respiration of cottonseed.

The average respiration intensity of cottonseed as a function of the moisture content of the seeds is plotted in figure 4. It is evident that the values for (RI) in any one series of seeds are smooth functions of the moisture content. It is to be expected that the vigor of the seeds and the general state of their enzymatic activity will differ for different samples of cottonseed, since differences in the conditions of growth and maturation may greatly affect the biological activity of the seeds. It is not surprising, therefore, that the seeds in one series (fig. 4) were found to respire at a different level from those in the other two series.

The difference in the behavior of the seeds in the 100 series and in the

200–300 series cannot be attributed to any significant difference in the weight of the kernels. An examination of representative samples of all three series gave values for the kernels as 56.9, 54.6, and 56.2 per cent. for the 100, 200, and 300 series, respectively.

It is apparent that once the seeds have matured, any additional time that they may spend in the boll before being harvested and ginned has no effect on their average respiration intensity. This fact is particularly clear in the results upon the 300 series where seeds of various ages are seen to fall on the same average respiration-intensity curve. It is also evident that there is no intrinsic difference between the respiration of the seeds that experienced an artificial addition of moisture and those in which there was a natural development of moisture.

The two samples, 204a, and 204b, which fell on neither of the two curves in figure 4, exhibited relatively high average respiration intensities. Both of these samples were from bolls that had been prematurely cracked open and had high moisture and free fatty acid contents when received (table I). Sample 204 was apparently the only lot that consisted of seeds that had not completely matured before being picked, a fact reflected in its respiration behavior. Measurements which will be reported in subsequent papers of this series demonstrate that this seed sample differed from the others in still other biological properties. Even though the seeds of samples 104 and 304 had likewise been obtained from prematurely cracked bolls, they, unlike those in 204a and 204b, had evidently already reached maturity in the bolls and, therefore, did not differ in respiration behavior from the seeds obtained from open bolls.

Comparison of cottonseed with other oilseeds

It is of interest to compare the respiration of cottonseed with that of other oilseeds. Larmour *et al.* (10) measured the daily respiration rates of flaxseed and sunflower seed samples of various moisture contents for periods up to 22 days after the seeds were conditioned to their final moisture levels. From their data, it is possible, therefore, to calculate the average

TABLE V

Values for average respiration rates of flaxseed and sunflower seed as calculated from the data of Larmour *et al.* (10)

Sample	Moisture content	Average respiration rate* (RI)
	%	*cc.*
Flaxseed	12.8	0.023
	13.7	0.064
	16.5	0.19
Sunflower seed	11.8	0.039
	13.2	0.073
	14.0	0.15

* Cubic centimeters of CO_2 per gram per day.

respiratory intensity of flaxseed and sunflower seed by the procedure described above. The results are shown in table V. A comparison of these values with those obtained for cottonseed (table IV) indicated that the flaxseed samples respired at a rate very close to that of the 200 and 300 series of cottonseed samples and the sunflower seed samples respired at a rate similar to that of the 100 series.

Quantitative expression of the effect of moisture on the respiration of cottonseed

Bailey (5) was able to convert the exponential type of curves which express the relationship between moisture content and respiratory rate into straight lines by the use of the equation:

$$y = ae^{bx} + c \qquad (2)$$

where y is the rate of respiration, x is the moisture content, and a, b, and c are constants characteristic of the material investigated. According to this

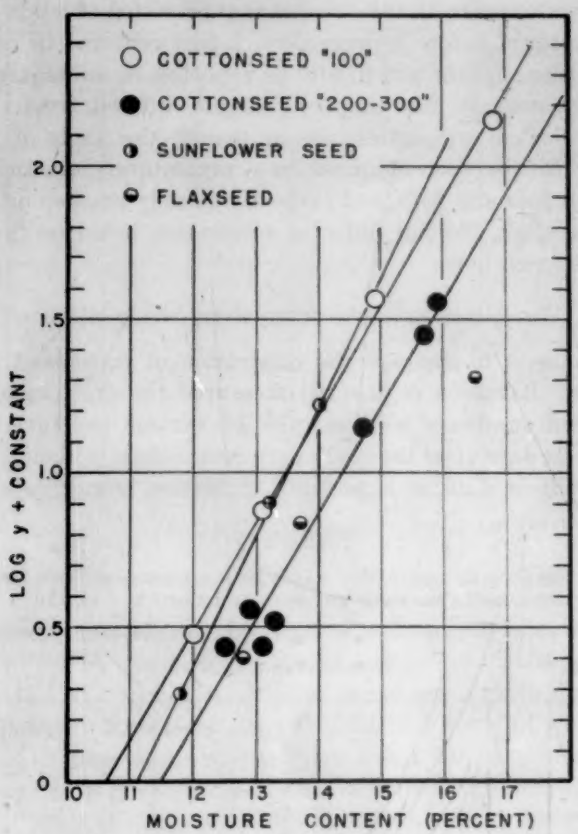

Fig. 5. Variation of the logarithm of the respiratory intensity (RI) as a function of moisture content of cottonseed, flaxseed, and sunflower seed.

equation, when log (y–c) is plotted against the moisture content, x, a straight line is obtained. The slope of this line, b, is, therefore, a quantitative expression of the effect of moisture on the rate of respiration of seeds. Bailey was unable to use the more simple exponential relationship,

$$y = ae^{bx} \qquad (3)$$

because when log y was plotted against x, a curve was obtained instead of a straight line.

When, however, the data for the average respiration intensity (RI) of cottonseed as presented in table IV were plotted according to equation (3), a straight line was obtained for values of moisture content in the range of 12 to 17 per cent. The straight lines obtained by this method are shown in figure 5. It will be noted that the lines representing the data of the 100 and the 200–300 series have the same slope. Thus the seeds in all of the series exhibit an increase in log y of 0.35 unit for a 1 per cent. increase in the moisture content. The only difference in the behavior of the 100 series and the 200–300 series is that the latter series is displaced 1 per cent. on the moisture axis.

Included in figure 5 are comparable average respiration intensity data for the flaxseed and sunflower seed samples of table V. The points for the sunflower seed samples fall close to those for the 100 series cottonseed samples. A straight line drawn through the points for the sunflower seed samples has a slope of log y equal to 0.52 unit per 1 per cent. increase in moisture content. The points for the flaxseed samples do not fall on a straight line. Two of the points, however, fall very close to the straight line representing the respiration of the 200–300 series cottonseed samples. It is significant that all three types of oilseeds respire at very similar levels of intensity and that the rate of increase of the logarithm of the respiration intensity with moisture content falls within the range of 0.35 to 0.42. It would be of advantage to extend this method of analysis and observation to other types of seeds.

Discussion

It is interesting to speculate on the nature of the relationship that exists between the moisture content of seeds and their respiration intensity. Bailey (3) has stated that a possible explanation for the increased respiration intensity with increased moisture content is that increase in moisture serves to reduce the viscosity of the gels in the seeds and thus to increase the rate of diffusion of the cell constituents. His position is best illustrated by means of following quotation:

> To restate, the production of heat is dependent upon the activity of the oxidizing enzymes of the kernel, the complex phenomenon being known as respiration. The latter is accelerated by an increase in the rate of diffusion, which in turn is dependent upon the existence of a gel, and relative viscosity of that gel. For these reasons the moisture content of sound grain determines to a considerable extent the rate of respiration and consequent liability of heating when bulk grain is stored.

In the light of newer knowledge concerning the activation and inhibition

of enzymes and of the mode of action of the enzymes within the cells, Bailey's thesis can be extended to include an explanation of the effect of moisture content on the activity of the enzymes. In dormant seeds, the food reserves remain practically unchanged during storage, even though there are present in these seeds the enzymes which are able to catalyze transformations in these substrates. In order to explain the phenomenon whereby enzyme and substrate coexist in seeds without the substrates being affected, it has been assumed that water is not distributed equally throughout the cell. There exist regions in the cell where both substrate and enzyme are in aqueous solution. These are regions of intense biological activity in which the more vigorous oxidation and hydrolytic activities take place. On the other hand, there are "islands" in the cell that are relatively water-poor. In such regions it would be expected that there would be very little oxidative or hydrolytic activity. KURSSANOV (**9**) has proposed that these "islands" are the seats of synthetic activity. It is highly probable that these regions are not sharply divided from each other and the change from a region of homogeneous solutions to a relative dry and non-homogeneous region is a gradual one accompanied by gradual change in the nature and degree of enzymatic activity.

In some of the first work on catalase, LOEW (**11**) noted that a portion of the enzyme in the tissue was not easily extractable with dilute buffers. It is well known that many enzymes are extractable from tissue only after the tissue has undergone an autolysis with the resultant disintegration of the tissue. Thus it is clear that the effective concentration of an enzyme in a cell depends not only on the total amount of enzyme present in the cell, but also on the manner in which the enzyme is bound to the tissue material. Unquestionably, much of the enzyme material in dormant seeds is rendered ineffective as a catalyst by the surrounding tissue.

Many types of enzymes are active only in the presence of reducing agents (**6, 16**) or other activators; *e.g.*, cyanide in papain-type enzymes (**7**). It is very likely, therefore, that the inactivity of some of the enzymes in dormant seeds is due also to the unavailability of activators.

On the basis of the above discussion, the effect of increased moisture content upon the respiration of seeds may be depicted provisionally as a sequence of the following events:

1. The immediate effect of increased moisture content in the cell is to increase the amount of active, readily soluble enzyme, and the amount of readily soluble substrate in the aqueous phase. This change is reflected in an increased respiratory activity within a short period after the moisture content has been raised.

2. Concomitant with the increase of the concentration of respiratory enzymes in the aqueous phase in the cell, there is also an increase of those enzymes which hydrolyze the food reserves and thus make possible the continued production of additional substrate for the oxidation reactions. Other types of hydrolytic enzymes which catalyze the disintegration of

cellular tissue are also activated, with the result that more enzymes are released into the aqueous phase.

3. The release of oxidative enzymes due to hydrolysis of the cellular tissue is reflected in an accelerated increase in respiratory activity.

4. The stimulated metabolic activity results in the formation of reducing agents and other activators which will still further increase the amount of active enzyme in the cells.

5. On the other hand, the fact must be recognized that, inasmuch as the respiration of resting seeds is not accompanied by synthetic activity, it is a very unproductive process. Instead of the energy of respiration being utilized in a furtherance of synthetic activity, most of it is released as heat. Those energy-rich compounds (such as phosphates, adenosine triphosphate, *etc.*) produced as a result of oxidation reactions, which would ordinarily be utilized in synthesis, will accumulate in resting seed and will act as inhibitors to further oxidation of the substrate.

6. Eventually a steady-state will be reached wherein the acceleration in respiratory activity due to the increase in the amount of substrate and enzymes released into the aqueous phase is balanced by inhibition due to the accumulation of products of respiration. This condition corresponds to the maxima in the respiration patterns observed in many of the cottonseed samples which were investigated.

7. Finally, the thermal destruction of enzyme activity, which takes place to greater extent in the aqueous phase than when the enzyme is adsorbed on cellular material, will result in an eventual diminution of respiratory activity.

The above description will explain the respiration patterns shown in figures 1, 2, and 3. Unquestionably, the seed lots that did not exhibit maxima in their respiration patterns would have developed them if the length of the storage interval had been sufficiently increased. Inasmuch as increase in moisture content has the effect of increasing not only the activity of existing active respiratory enzymes in the seeds, but also the rate of production of additional active enzymes, it can be understood why the respiratory intensity might be expected to be an exponential rather than a linear function of the moisture content.

At any given moisture content, the respiratory intensity will be dependent on the vigor of the seeds; *i.e.*, on the total amount of enzyme available for catalytic activity and the partition of the total enzyme between the active aqueous phase and the relatively inactive adsorbed phase. The differences between the seeds in the 100 and in the 200 and 300 series could be due either to a difference in the vigor of the seed or to a difference in the structure of the cells. Differences in cell structure could be expected to affect the equilibrium between the active and inactive forms of the enzyme.

Although in the foregoing discussion the respiration of cottonseed was attributed to enzymatic activity rather than to the activity of the microorganisms associated with the seeds, it is true that the observed phenomena

might just as well have been explained as due to the activity of bacteria and molds. Experimental evidence is not contained in this paper to permit evaluation of the relative rôles played by micro-organisms and seed enzymes in the respiration of cottonseed. Evidence is presented in another paper of this series (**2**) which indicates that most of the biological activity observed in cottonseed, under the described conditions of moisture content and temperature, is due to seed enzymes.

Summary

1. The pattern of respiration has been determined for 17 lots of cottonseed with moisture contents ranging from 10 to 17 per cent. The respiratory quotient (RQ) of resting cottonseed was found to be unity.

2. The intensity of respiration of resting seed (RI) has been defined as the average rate of respiration of the seeds over a period exceeding 100 days.

3. The average respiratory intensity of cottonseed is an exponential function of the moisture content of the seed. Mature seeds of the "Delfos" variety respired at a higher level than did the seeds of the "Coker's" or "Oklahoma Triumph" varieties.

4. The exponential-type curves which express the relationship between moisture content and respiratory intensity can be converted into linear curves by the use of the equation:

$$y = ae^{bx}$$

where y is the average respiration intensity (RI), and x is the moisture content. The value of b is 0.35 unit per 1 per cent. of moisture content for all samples of cottonseed investigated.

5. The values for the logarithm of the respiratory intensity for the "Coker's" and "Oklahoma Triumph" samples can be made to coincide with the line representing values for the "Delfos" samples by shifting them one per cent. on the moisture axis.

6. The respiratory intensity of sunflower seed samples calculated from the data of LARMOUR, *et al.* (**10**) can be analyzed in the same manner used for the cottonseed samples. Sunflower seed behaves in a manner very similar to the "Delfos" variety of cottonseed. The value of b for sunflower seed is 0.42 unit per 1 per cent. increase in moisture content.

7. The respiratory intensity of flaxseed samples as calculated from the data of LARMOUR, *et al.* (**10**) is very similar to that of the "Coker's" and "Oklahoma Triumph" varieties of cottonseed.

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STABILITY OF THE BUFFER SYSTEM OF LEMON JUICE[1]

WALTON B. SINCLAIR AND DESIRE M. ENY

(WITH FIVE FIGURES)

In previous studies (**11, 12, 13**), the authors have shown that the buffer systems of lemon and grapefruit juices are similar to those of pure citric acid solutions which contain amounts of free acid and salts (combined acids) in amounts equivalent to those of the juices. Consequently, the titration curves of lemon and grapefruit juices differ slightly from a pure citric acid solution provided a correction is made for the salts (combined organic acids) naturally occurring in the juices. Since these curves are nearly identical in slope, it can be reasonably assumed that the stability of the buffer system of lemon juice under various conditions is similar to that of a pure citric acid solution and its salts. To prove this hypothesis, experiments were planned to study the effects of certain factors, which as dilution, various amounts of neutral salts, sucrose and heat on the stability of the buffer properties of both lemon juice and citric acid–citrate solutions. The stability factor is measured in terms of the changes in pH of the systems under the different treatments.

Citrus juices have an efficient buffer system as shown by the fact that they resist changes in pH when hydrogen or hydroxyl ions are added to them. As citrus juices are naturally high in acidity, their maximum buffer capacity occurs on the acid side. All biological fluids are, to some degree, buffered against sudden changes in pH when strong acids or bases are added to the systems. This type of mechanism is essential for stabilizing the reaction media which are necessary for the normal function of living tissues.

Materials and methods

The data in this investigation were obtained on pure citric acid solutions and lemon juice. A weighed amount of anhydrous citric acid was used for the solutions in which known volumes of standard NaOH were added to form the desired amount of salt with a common anion. The lemon juice samples were obtained from immature yellow lemons. Centrifuged juice was used in all experiments. The free acid content of the juices and that of the citric acid solutions were determined on aliquots by titration with phenolphthalein as indicator. The pH measurements were read on a Beckman pH meter. The combined acids of various juice samples were estimated by determining the alkalinity of the ash from an aliquot of juice. A known amount of standard HCl was added to the juice ash and the excess acid was titrated with NaOH. The alkalinity was calculated from the equivalents of HCl neutralized by the ash. This value represents closely the equivalents of combined acids present in the original sample of juice.

[1] Paper no. 541, University of California Citrus Experiment Station, Riverside, California.

The concentrated juice samples were prepared in vacuo at 50° C. The effect of heat on the buffer system of the juice was determined on samples previously boiled for 30 minutes over a free flame and then made to the original volume with distilled water. The effect of sucrose was determined by adding weighed amounts to samples of lemon juice and to citric acid solutions. In the experiments where larger amounts of sucrose were added, the samples were slightly heated over a free flame for complete solution. The neutral salt solutions of KCl, NaCl, K_2SO_4 and KNO_3 were made to 1 normal. Volumetric increments of these solutions respectively were added to the lemon juice samples.

Results

Effect of dilution on the pH of lemon juice

According to the so-called Henderson-Hasselback equation (3) the pH

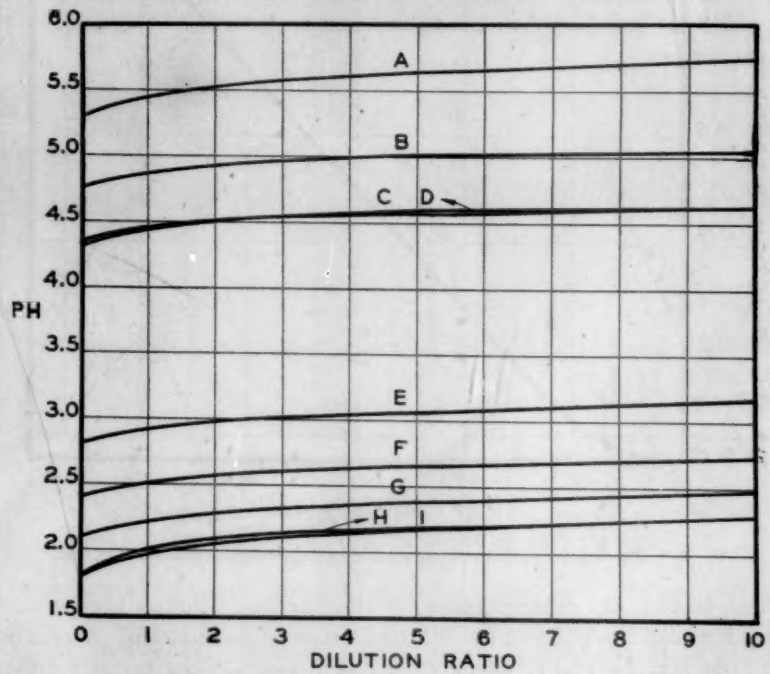

Fig. 1. Changes in pH with the dilution ratio of citric acid systems containing different amounts of salt. Curves A, 60 mg./ml. citric acid—80 per cent. salt; B, Lemon juice containing 41.92 mg./ml. of acid (as citric)—63 per cent. salt; C, Lemon juice containing 41.92 mg./ml. of acid (as citric)—50 per cent. salt; D, 60 mg./ml. citric acid—50 per cent. salt; E, Lemon juice containing 41.92 mg./ml. of acid (as citric)—13 per cent. salt; F, Lemon juice containing 41.92 mg./ml. of acid (as citric)—no salt added; G, 60 mg./ml. citric acid—3.2 per cent. salt; H, 375 mg./ml. citric acid—4.78 per cent. salt; I, Concentrated lemon juice 360 mg./ml. (as citric)—no salt added.

of solutions of weak acids and their salts are not affected to any great extent by dilution. In such systems, the dissociation constant and the ratio of the acid to salt determine the approximate pH; and, since this ratio is not

changed by dilution, a relatively small change in pH occurs. This relationship is only approximate and sufficient for most practical purposes, but it is not entirely true. In addition to the ratio of acid to salt, the total concentration of ions in the system is involved in the pH changes of a buffer solution on diluting with water. The activity coefficients change with the ionic strength. At greater ionic strengths than 0.1 normal, the difference between the individual ions in the system becomes so great as to prohibit the use of an average activity coefficient. Pure solutions of weak acids can undergo considerable dilution without any great change in pH provided the dissociation constant (Ka) is small and the undissociated residue sufficiently high to keep Ka constant.

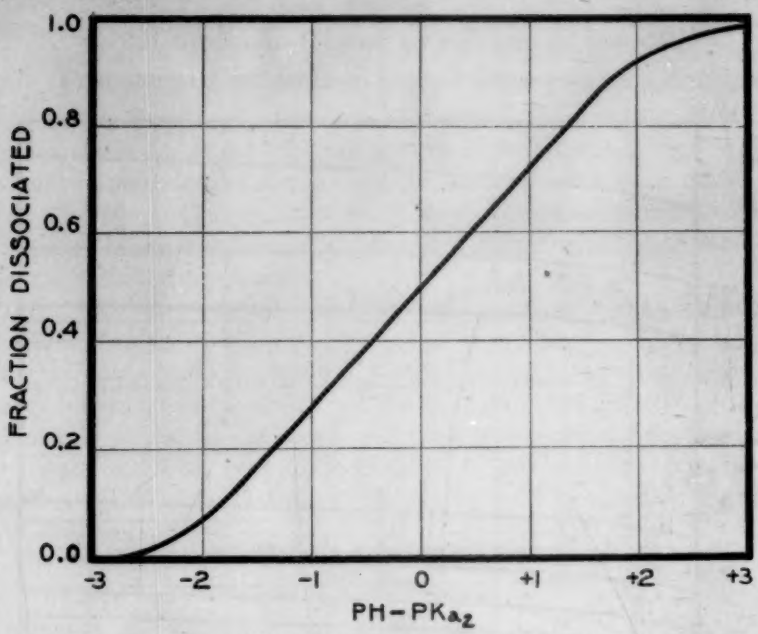

FIG. 2. The degree of dissociation of lemon juice as a function of pH.

These factors are demonstrated by the curves of figure 1 showing the relation between the pH and the dilution ratios of normal and concentrated lemon juice samples and of various citric acid solutions containing different amounts of salt. The total acid concentration and the ratio of salt to acid appear to be the important factors affecting the change in pH on dilution of lemon juices and citric acid–citrate solutions. As the difference in concentrations of the acid and salt increases in these systems, the buffer capacity is reduced and a slightly greater increase in pH occurs on dilution. A citric acid solution (curve A) containing 60 mg. per ml. citric acid and 80 per cent. salt showed a change in pH of 0.45 when diluted to ten times its volume. Another citric acid solution (curve H) containing 375 mg. per ml. and 4.78 per cent. salt showed a change in pH of 0.47 when diluted to ten times

its volume. A concentrated lemon juice (curve I) containing 360 mg. per ml. citric acid changed, under similar conditions, 0.47 of a pH. Under these experimental conditions, curves H and I are practically identical, and the changes in pH of lemon juice, when diluted or concentrated, are similar to those of a pure citric acid–citrate solution of equivalent concentrations. Although natural lemon juice has a substantial buffer capacity, this system is not at a maximum as the ratio of free acid to salt is far from unity.

Curves C and D are practically identical and they show a maximum change in pH of 0.28 when a lemon juice sample (41.92 mg. per ml. citric acid and 50 per cent. salt) and a citric acid solution (60 mg. per ml. citric

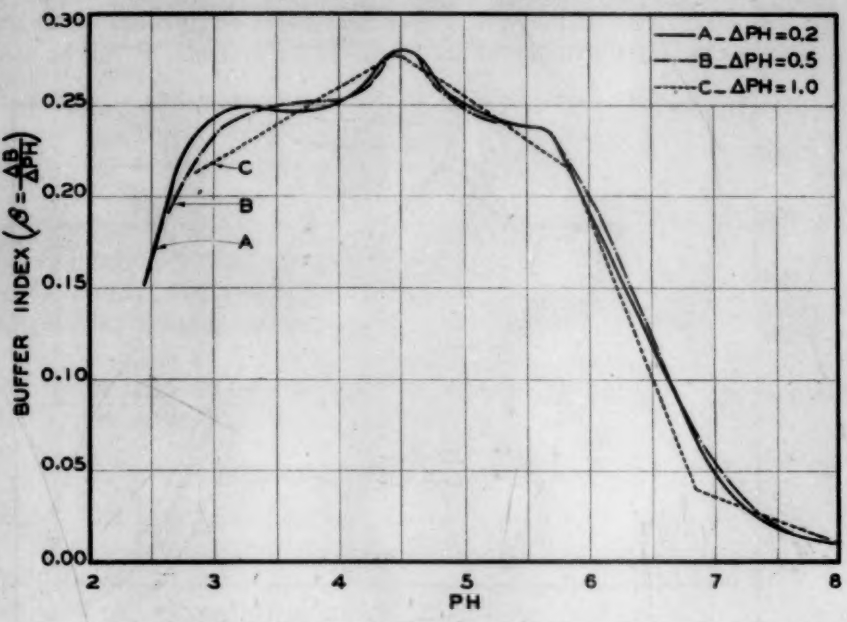

FIG. 3. Buffer index curves of a lemon juice sample expressed as a function of pH. Each curve was constructed with a different increment of ΔpH.

acid and 50 per cent. salt) are diluted to ten times their volumes with water. In these samples, the ratio of free acid to salt is approximately equal to one; the buffer capacity is then at a maximum, and the change in pH occurring upon dilution is at a minimum. Curves B, E, F and G (fig. 1) show the variations of pH on dilution where the salt/acid ratio deviates from 1.

The effective buffer range of lemon juice on both sides of pKa_2 is shown in figure 2. This curve is similar in shape to the fundamental curve generally ascribed to weak acids and their salts (3). When 50 per cent. of the acid of lemon juice is in the salt form (or dissociated), the pH is equal to pKa_2. At this point the juice has a maximum buffer capacity and the ratio of free acid to salt is unity. The buffer range of lemon juice is represented by the straight portion of the curve and extends over an approximate range of $pH = pKa_2 \pm 1.8$.

Buffer index of lemon juice

The buffer properties of lemon juice are more accurately indicated by the buffer index curves of figure 3. These curves represent the reciprocal of the slope of the titration curve (see fig. 5) when the amount of base used in the titration is expressed in equivalents. If the pH values are plotted on the abscissa, the buffer index measures the degree of slope of the titration curve. The buffer index is mathematically expressed by the differential ratio $\frac{dB}{dpH}$, where dB is an infinitesimal part of an equivalent of base, and dpH is an infinitesimal change in pH. The buffer index curves (fig. 3, curves B and C) calculated with larger increments of ΔpH shows trends rather than accurate representations of the buffer system. It can be seen

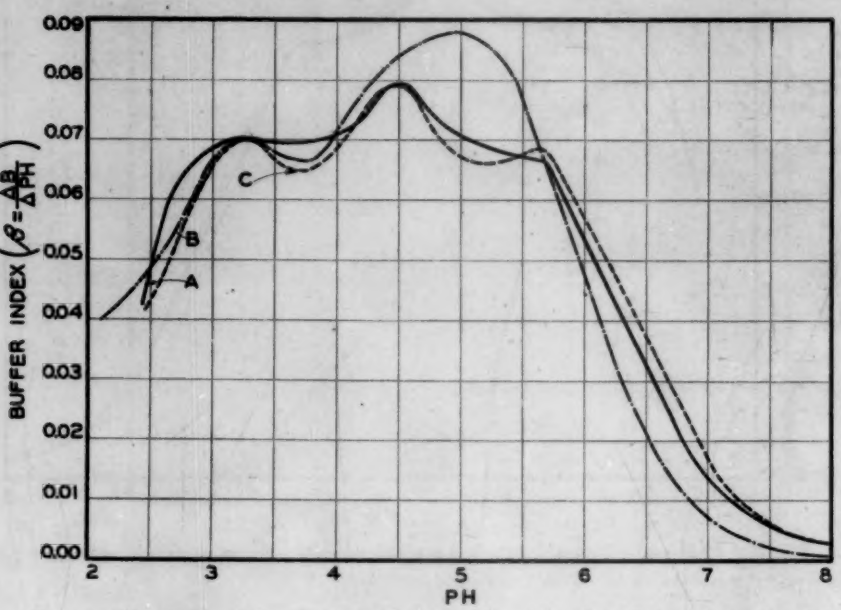

Fig. 4. The buffer index curve of lemon juice (A) plotted in terms of a 0.1 M solution, compared with the theoretical curve (B) of Hastings and Van Slyke (6) for 0.1 M citric acid solution, and with the experimental curve (C) for citric acid of 0.1 M concentration.

that the smaller the increment of change in pH (ΔpH) the more nearly the experimental curve approaches the real buffer index curve of lemon juice. The three curves were determined on the same sample of juice, but each one was plotted from values calculated with increments of ΔpH of 0.2, 0.5 and 1.0, respectively. Curve A is calculated from the data using ΔpH = 0.2. Note that the maximum buffer capacity of lemon juice is at a pH of 4.4 which is in close agreement with the dissociation exponent of the second dissociation constant of citric acid ($pKa_2 = 4.39$–4.50). Curve A also shows in detail that the buffer capacity of lemon juice is between pH 2.7 and 5.6. The two minor inflections indicate the position of pKa_1 and pKa_3.

The position of the curves on the graph, with respect to the buffer index values, is influenced by the concentration of the free acid in the lemon juice samples. In other words, the buffer capacity of a solution is governed by the concentration of the components. As the buffer index cannot be represented by a constant, it must be defined in terms of a given pH. This particular lemon juice sample had a total acidity of 68.16 mg. per ml. of citric acid and citrate. This is equivalent to a 0.355 molar solution in terms of the total citric acid radicle in the sample. The maximum buffer index (β) of the lemon juice was 0.279 at pH 4.4. By simple calculation from these data, the maximum molar buffer index (β_M) of lemon juice at pH 4.4 would be 0.79. According to VAN SLYKE (**14**), citric acid has a maximum β_M of 0.84. The β_M of lemon juice agrees surprisingly well with that of pure citric acid solutions.

The agreement of the β values for lemon juice with those of a citric acid solution are further illustrated in figure 4. The buffer index values for lemon juice (curve A) are plotted in terms of 0.1 M concentration. This was done so a comparison could be made with the theoretical curve of HASTINGS and VAN SLYKE (**6**) for 0.1 M citrate solutions (curve B). Their curve was estimated and constructed by graphic summation from the three pKa values of citric acid. The distinct difference between these two curves is in the position of the maximum buffer index which is at pH 5.0 for the theoretical curve and at pH 4.4 for the lemon juice. On the contrary, the experimental curve obtained for 0.1 M solution of citric acid (curve C) agrees closely with curve A for lemon juice.

EFFECTS OF NEUTRAL SALTS ON THE PH OF LEMON JUICE

Although most biological systems are buffered, the reactions occurring in the tissues are carried out in relatively high concentrations of mixed electrolytes. In plant tissue fluids, changes occurring in pH are not accompanied by corresponding changes in total salt concentration. In lemon juice and other citrus fruits, the organic acids are slightly ionized, and the inorganic salts that affect the pH are those that liberate free cations during the metabolism of the fruit. One important characteristic of the buffer systems of citrus juices is that their total salt or ionic concentration can be reduced by dilution, and at the same time, the system maintains an adequate buffer intensity. Cells of plant tissues, in general, can become hydrated, thus decreasing the effect of relatively high concentrations of electrolytes, and maintain a constant reaction medium with respect to changes in hydrogen-ion concentration.

The data in table I show the effect of different concentrations of neutral salts on the pH of lemon juice. In concentrations as high as 10 m.e. per ml. of lemon juice, KCl and NaCl produced no significant change in pH. With the same concentrations of K_2SO_4 or KNO_3, increases in pH of 0.18 and 0.19, respectively, were recorded. Although no reasons are given for these results, HAYNES and BROWN (**7**) report a similar effect of K_2SO_4 on apple

TABLE I

EFFECT OF NEUTRAL SALTS ON LEMON JUICE

NEUTRAL SALTS ADDED TO JUICE	pH OF LEMON JUICE (43.68 MG./ML. FREE ACID (AS CITRIC)) WITH VARIOUS AMOUNTS OF NEUTRAL SALTS			
M.E./ML.	KCl	K_2SO_4	NaCl	KNO_3
0.0	2.32	2.32	2.32	2.32
0.68	2.32	2.32	2.32	2.32
1.37	2.32	2.33	2.32	2.32
2.05	2.31	2.34	2.31	2.32
2.73	2.31	2.35	2.31	2.33
3.41	2.31	2.37	2.31	2.34
4.10	2.31	2.40	2.31	2.35
4.70	2.31	2.40	2.31	2.35
5.46	2.31	2.42	2.32	2.37
6.14	2.31	2.44	2.32	2.41
6.83	2.31	2.46	2.32	2.43
8.00	2.31	2.48	2.32	2.47
9.00	2.32	2.49	2.33	2.49
10.00	2.32	2.50	2.33	2.51

juice. If either the cations or anions of the neutral salts had reacted chemically with some substance in the lemon juice, a shift in pH of the sample would have occurred. The different neutral salts produced no marked effect on the pH of lemon juice. This is evidence that the neutral salts remained in ionic form. It is generally recognized that salts of strong acids and strong bases are neutral in aqueous solutions. As 85 per cent. of the total ash of the edible portion of the lemon is soluble in the juice, it is highly probable that most of the ash constituents exist in the ionic state. The cation-anion balance of the neutral salts does not influence the alkaline ash and pH of the juice. It is the excess cations that are available for salt formation with the organic acids which affect the pH.

EFFECT OF SUCROSE ON BUFFERED SOLUTIONS

The soluble solids of citrus fruits are chiefly composed of soluble sugars and organic acids. In biological systems, the sugars play an important rôle in the osmotic activity of the cells, but produce no appreciable effect on the pH. The dissociation constants of the sugars are of such magnitude (of the order of 10^{-13}) that they are probably without buffer effect in citrus juices. In table II are recorded the pH values of different buffered solutions containing various amounts of sucrose. The first two samples are lemon juices, and the others are citric acid solutions buffered at different pH values by varying the salt content. No significant change in pH occurred in any of these buffered systems that contained a concentration of sucrose as high as 50 per cent. As sucrose forms a hexahydrate in water (**9**), one mole of sucrose dissolved in 1000 grams of water would combine with six moles of water, leaving 892 grams of water for solvent purposes ($1000 - (18 \times 6) = 892$ grams). In the presence of lyophilic colloids the amount of water taken up by sucrose varies (**5**). The small amount of lyophilic colloids present in

TABLE II

EFFECT OF SUCROSE ON LEMON JUICE AND BUFFERED SOLUTIONS

TYPE OF BUFFER SOLUTION	pH OF VARIOUS SOLUTIONS CONTAINING DIFFERENT PERCENTAGES OF SUCROSE						
	0	5	10	15	20	25	50
Fresh lemon juice—33.34 mg./ml. free acid (as citric)	2.43	2.43	2.43	2.43	2.43	2.43	2.43
Concentrated lemon juice diluted 1 to 5—60.16 mg./ml. free acid (as citric)	2.18	2.18	2.18	2.18	2.18	2.18	2.18
Citric acid—60 mg./ml. + 3.2% salt*	2.12	2.12	2.12	2.12	2.12	2.12	2.12
Citric acid—60 mg./ml. + 6% salt	2.40	2.40	2.40	2.40	2.40	2.40	2.39
Citric acid—60 mg./ml. + 80% salt	5.34	5.34	5.34	5.34	5.34	5.34	5.32
Citric acid—60 mg./ml. + 50% salt	4.33	4.33	4.33	4.33	4.32	4.32	4.32
Citric acid—360 mg./ml. + 3.1% salt	1.70	1.70	1.70	1.70	1.70	1.70	1.69
Citric acid—360 mg./ml. + 35% salt	3.40	3.40	3.40	3.40	3.40	3.41	3.40

* A quantitative amount of NaOH was added to obtain the desired percentage of salt.

centrifuged lemon juice indicates that the amount of water available for solvent purposes would not be affected. Under these experimental conditions, the only means by which sucrose could produce a change in pH of lemon juice is by reducing the apparent volume of the solvent of the system. By so doing, the acid and salt constituents that affect the pH of the juice would be increased accordingly. The ratio of free acid to salt, however, would not be changed. The amount of water used to form the hexahydrate in a 50 per cent., by weight, sucrose solution would be approximately 15 per cent. Such a small reduction in volume would hardly cause a significant change in pH.

EFFECT OF HEAT ON THE BUFFER CAPACITY OF LEMON JUICE

Citric and malic acids in aqueous solutions are chemically stable at boiling temperatures. Since the buffer capacity of citrus juices is due chiefly to these nonvolatile organic acids and their salts (**10, 11**), it is reasonable to presume that the pH of these juices should not undergo any great change at the boiling point and at atmospheric pressure. BARTHOLOMEW and SINCLAIR (**2**) have already shown that the course of the titration curve for orange juice that was boiled for 45 minutes was similar to that for normal juice. The slightly higher pH value of the boiled juice showed that this juice required slightly less NaOH than unboiled juice for neutralization. The titration curves of juice samples, boiled for 5, 10, 15 and 30 minutes, respectively, were so close to those of normal juice that it was impractical to put them on the same graph. As the ratio of the free acid to salt is much greater in the lemon than in the orange, it was decided to compare the titration curves of boiled and normal lemon juice for the purpose of measuring the effect of heat on the buffer capacity of this system. This type of experiment should show whether the heat decomposed or volatilized any of the acid constituents and thus lessened the amounts of NaOH required for

neutralization. It should show, also, the effect of heat on the soluble pectins or on any other constituents that might hydrolyze and thus increase the amounts of NaOH required for neutralization.

The effect of heat on the buffer system of lemon juice is amply demonstrated by the titration curves for normal lemon juice (fig. 5, curve A) and for a portion of the same juice boiled for 30 minutes (fig. 5, curve B). For comparative purposes, a titration curve is drawn of pure citric acid with a concentration of free acid equivalent to that of lemon juice at its natural pH. The course of the titration curve of lemon juice is not affected

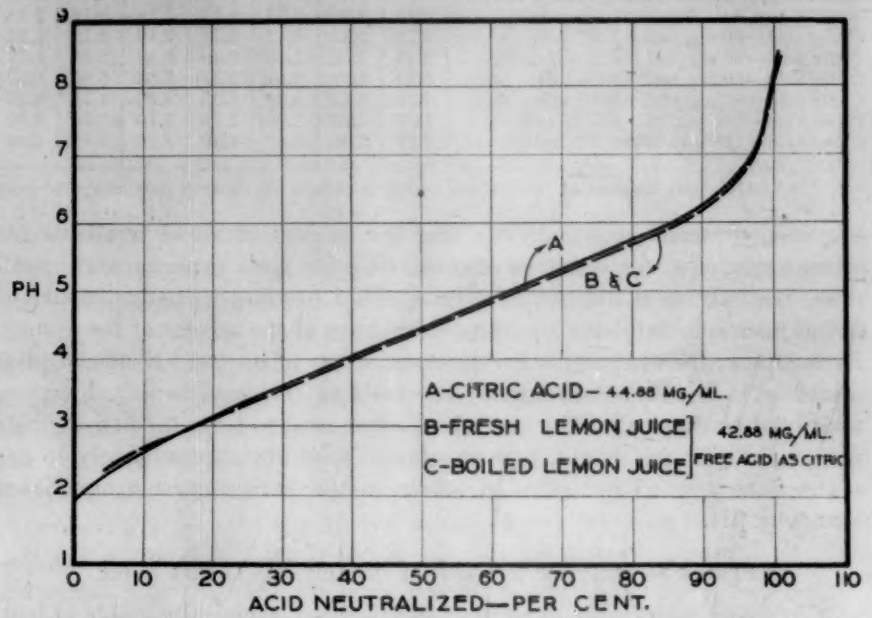

FIG. 5. Titration curves of lemon juice normal and boiled and pure citric acid solution. One curve was drawn for B and C as their values were too close to separate graphically. A correction was made on the curves B and C for the salt naturally occurring in the juice.

by heat. There is no significant difference in these three curves at any given pH between 0 and 100 per cent. neutralization. These results agree with those of DUNNE (4) who showed that boiled and filtered sap of wheat and buckwheat plants gave titration curves identical with those of the fresh sap; of BARNES (1) who observed that the hydrogen-ion concentration was only slightly lower in cooked apple juice than in fresh; and of MARSH (8) who showed that the buffer capacity of juice of nonacid vegetables was affected only slightly when heated in acid solution.

Summary

The buffer system of lemon juice is similar to that of citric acid–citrate solutions of equivalent concentrations. In lemon juice samples and pure

citric acid solutions, the increase in pH with the dilution ratios is influenced by the concentrations of total acid and salt. The smallest change in pH on dilution occurred in systems where the ratio of free acid to salt was unity. As lemon juice contains a large ratio of free acid to salt, the changes in pH are relatively large with increase or decrease in juice volume. The increase in pH with dilution is approximately the same for lemon juice and pure citric acid solutions of equivalent concentrations. The efficient buffer capacity of lemon juice extends over an approximate range of $pH = pKa_2 \pm 1.8$. This agrees with the maximum range of the buffer index curve. The buffer index value of lemon juice is at a maximum at pH 4.4. This value is in agreement with the pKa_2 of a pure citric acid solution. The two minor inflections on the curve indicate pKa_1 and pKa_3 values of lemon juice. In concentrations as high as 10 m.e. per ml. of lemon juice, KCl and NaCl produced no significant change in pH. With the same concentrations of K_2SO_4 and KNO_3, increases in pH of 0.18 and 0.19, respectively, were recorded.

In concentrations as high as 50 per cent. by weight, sucrose did not change the pH values of various lemon juices and citric acid solutions which had different ratios of free acid to salt.

That the buffer capacity of lemon juice is not affected by heat is demonstrated by the fact that boiled and normal juices have identical titration curves.

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CHANGES IN THE COMPOSITION OF SQUASH DURING STORAGE[1]

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Studies by LE CLERC DU SABLON in 1905 (**5**) established the general nature of the changes occurring in the fruits of Cucurbits during maturation and storage. At maturity the solids of the fruits of *Cucurbita pepo* var. courge olive were high in starch and relatively low in sugar. During storage the conversion of starch to sugar was rapid, as was also the loss of sugar by respiration. On long storage much of the total carbohydrate had disappeared and the water content of the fruit had increased markedly.

Later reports have added detail to these findings and have served to show the great variations in composition among the many varieties of the species commonly called pumpkin or squash. This work has been reviewed and greatly extended in the recent paper by CULPEPPER and MOON (**2**).

Members of the staff of the New Hampshire Agricultural Experiment Station, under the leadership of the Department of Horticulture, have been engaged for some years in a study of the problems connected with the storage of squash. During three seasons samples of three varieties of squash have been taken at harvest and after various periods of storage. It was expected that the chemical analysis of these samples would furnish more detail, and especially more exactly quantitative information, about changes during storage than has been available.

Material and methods

The varieties studied were Blue Hubbard and Buttercup, strains of *Cucurbita maxima,* and Butternut, a strain of *C. moschata.* The fresh weight of each sample was 150 gm., 30 gm. of which were taken from the edible portion of each of five fruits selected at random from a large lot of apparently uniform specimens. The samples were preserved in 80 per cent. alcohol and extracted and analyzed by essentially the same methods used in earlier studies of the timothy plant (**6**).

As preliminary tests showed that lignin is either absent or present in very small amounts, this determination was omitted. Total pectin was determined by a modification of the method used by DOTY *et al.* (**3**) as follows: A sample of the residue from the alcohol extraction of sufficient size (0.0625 to 0.2500 gm.) to yield not more than 0.0250 gm. of calcium pectate was extracted for four successive 30-minute periods with 40 ml. of boiling N/30 HCl. The filtrate and washings from each extraction were neutralized immediately to methyl red indicator. The combined extracts were diluted to 300 ml. in a 600-ml. beaker; 100 ml. of 0.1 N NaOH were added, and the solution was let stand overnight. It was then acidified with 50 ml. N CH_3COOH, after which 50 ml. M $CaCl_2$ were added. After allowing to

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stand until the flocculent precipitate had settled, much of the supernatant liquid was siphoned off, leaving the precipitate suspended in a volume of 100–150 ml. To this, 0.1500 gm. of dry asbestos was added, the mixture was boiled for a few moments, then filtered on an asbestos pad weighed in a Gooch crucible, and washed thoroughly with hot water, then with alcohol and finally with ether. Crucible and contents were dried in an oven at 102° C. and weighed. Duplicate portions of the asbestos, 0.1500 gm., were carried through the boiling, filtration, washing and drying to serve as blanks.

In the earlier samples starch was determined as the fermentable sugars produced from the alcohol extracted residue by hydrolysis with N H_2SO_4 for 2.5 hours, and also by the action of Taka diastase for 40 hours. The two methods agreed so closely that only acid hydrolysis was used with the later samples.

The absence of maltose from four of the samples of Blue Hubbard taken at various periods from harvest to six months storage was established by the following procedure. In one portion of the cleared extract total sugars were determined by the reducing power after treatment with invertase. In another portion the action of invertase was preceded by the same Taka diastase treatment that is used for starch. As the diastase treatment hydrolyzes maltose completely, the reducing power of the sample should be increased by this treatment if maltose were present. No increase was found in the squash samples. The same treatment, applied to the extract from the endosperm of germinating corn, indicated large amounts of maltose. The method appears conclusive as far as proving the absence of maltose is concerned. Studies of the use of this method for the quantitative determination of maltose in the presence of other sugars have not as yet been completed.

In sampling the 1943 harvest the loss in fresh weight of each fruit was determined from harvest until the time of sampling. These figures, with the analysis of the samples, made possible an estimate of the total change in amount of the various constituents during the storage period. A partial statement of these results has been reported in a general bulletin on storage problems (7). A more elaborate system was adopted for the 1944 harvest. About 35 fruits of each of the three varieties were kept in a separate storage room. The weight of each squash was recorded at regular intervals until it was used as a sample or until it spoiled. With the Blue Hubbard this was continued for six months. All the others had been lost before that time.

On sampling, the inner fiber and seeds were separated from each fruit. Their fresh weight was determined, and the parts from the five fruits were mixed and sampled. After extraction with 80 per cent. alcohol the seed samples were extracted with ether, and the ether-soluble materials in the alcohol extract were determined. The sum of these two items is listed as "ether extract" in the presentation of the results. Pectin appeared to be practically absent from the seed. The fiber samples were analyzed by the same methods used for the flesh.

Results and discussion

The composition of the edible portion of the squash is presented in table I. The figures are the averages of the samples taken at harvest and at various storage periods during three seasons. The amounts listed under "Total carbohydrates" are the sums of those for glucose, fructose, sucrose and starch, all calculated to their starch equivalent.

The results of earlier studies are confirmed in the following respects: (1) The rapid disappearance of starch; (2) the increase in sugars, at least to three months' storage; (3) the decrease in total carbohydrates; and (4) the increase in water content. It appears that the water content depends on the balance between the loss of water by evaporation and its gain by respiration. The Blue Hubbard lost fresh weight more slowly than did the other varieties. Its water content increased markedly, that of the Buttercup only slightly, and that of the Butternut not at all.

In a chemical study of the development of fruits of cucurbits ARASIMOVICH (1) reported glucose always in excess of fructose. In the present study fructose was found in excess of glucose in all samples taken at harvest. The proportion of glucose increased during storage. Table I indicates that Butternut is an exception in this respect, but in a single fruit which had been in storage for a year, glucose was 5.52 per cent. and fructose 3.25 per cent. of the dry weight.

This trend toward an increased proportion of glucose is opposite that found in haplocorms of timothy (4) in which carbohydrate is stored as fructosan. In that case it was suggested that relatively slow interconversion of glucose and fructose might explain the accumulation of fructose. The same statement could explain the accumulation of glucose in the present case, since the reserve carbohydrate is starch. The carbohydrate relationships are complicated by rapid respiration. At present there is, in this case, no direct evidence as to which sugar is used most rapidly in this process.

The relatively large amount of sucrose in Butternut after storage is striking. This may be one factor in the very desirable flavor of this variety. Since no maltose was found in such samples of Blue Hubbard as were tested for it, the participation of amylases in the conversion of starch to sugar seems doubtful. Phosphorylation would appear to be more probable. In percentage of dry weight, both cellulose and pectin increase during storage. Since total nitrogen increases also, it is possible that all these increases are relative rather than absolute.

The composition of the fiber differs in detail, but not strikingly, from that of the edible portion. During storage its changes in composition differ from those of the flesh, especially in the following respects: (1) There is an appreciable loss of water; (2) the loss of total carbohydrates is much more rapid; and (3) the gains in cellulose and pectin are much more pronounced.

The water content of the seeds is relatively high at harvest and decreases greatly during storage (table II). Total carbohydrates are low compared with other parts of the fruit, but they are considerably higher in the seeds

TABLE I

AVERAGE COMPOSITION IN PERCENTAGE DRY WEIGHT OF THE EDIBLE PORTION OF SQUASH AT VARIOUS STORAGE PERIODS. 1942–44

	Blue Hubbard				Buttercup			Butternut		
	Harvest	2 weeks	3 months	6 months	Harvest	2 weeks	3 months	Harvest	2 weeks	3 months
	%	%	%	%	%	%	%	%	%	%
Soluble solids	35.05	46.82	71.82	71.71	20.89	30.90	55.38	24.96	36.82	58.18
Glucose	9.71	14.53	25.30	30.04	3.52	7.34	16.85	2.18	2.68	0.63
Fructose	10.94	14.06	20.56	15.60	4.77	7.50	12.04	4.72	5.08	3.08
Total reducing sugars	20.65	28.59	45.86	45.64	8.29	14.84	28.89	6.90	7.76	3.71
Sucrose	3.56	5.07	10.84	6.65	4.43	7.46	14.42	8.07	18.23	39.84
Total sugars	24.21	33.66	56.70	52.29	12.72	22.30	43.31	14.97	25.99	43.55
Starch	42.32	28.54	4.41	0.48	53.44	43.32	14.54	52.86	40.57	18.48
Total carbohydrates as starch	64.01	58.96	55.76	47.68	64.36	63.29	54.16	66.94	64.43	59.41
Cellulose	5.82	7.52	9.50	13.15	5.59	6.50	10.27	6.43	6.95	8.02
Pectin	3.72	4.28	5.88	6.95	3.26	3.33	6.28	3.84	3.62	5.38
Total N as protein	6.14	6.08	6.26	8.17	5.22	5.35	7.09	6.54	6.94	7.80
Solids % F.W.	14.43	13.42	13.01	10.60	24.31	24.51	18.49	17.59	17.34	17.56

TABLE II

COMPOSITION OF THE SEED OF SQUASH AT VARIOUS STORAGE PERIODS IN PERCENTAGE DRY WEIGHT

	Blue Hubbard				Buttercup			Butternut		
	Harvest	2 weeks	3 months	6 months	Harvest	2 weeks	3 months	Harvest	2 weeks	3 months
	%	%	%	%	%	%	%	%	%	%
Total carbohydrates as starch	15.57	5.26	3.65	4.62	9.94	5.66	6.75	26.10	12.84	10.76
Cellulose	14.96	11.64	13.94	12.46	10.76	11.11	11.87	8.34	8.61	9.09
Ether extract	12.92	34.87	29.91	31.56	29.05	35.08	31.92	8.47	30.54	28.74
Total N as protein	23.21	28.99	28.72	31.29	27.26	29.72	28.39	21.09	29.40	31.16
Solids % F.W.	23.48	48.64	58.84	61.28	34.12	54.76	67.28	29.40	46.60	66.12

TABLE III

CONSTITUENTS IN GRAMS PER KILOGRAM FRESH WEIGHT AT HARVEST. AVERAGES, 1942–44

	BLUE HUBBARD				BUTTERCUP			BUTTERNUT		
	HARVEST	2 WEEKS	3 MONTHS	6 MONTHS	HARVEST	2 WEEKS	3 MONTHS	HARVEST	2 WEEKS	3 MONTHS
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Solids	144.3	129.9	117.5	89.0	243.1	221.1	143.0	175.9	154.1	131.6
Soluble solids	50.58	60.82	84.39	63.82	50.78	68.32	79.19	43.90	56.74	76.56
Glucose	14.01	18.87	29.73	26.74	8.56	16.23	24.10	3.83	4.13	0.83
Fructose	15.79	18.26	24.16	13.88	11.60	16.58	17.22	8.30	7.83	4.05
Total reducing sugars	29.80	37.13	53.89	40.62	20.16	32.81	41.32	12.13	11.96	4.88
Sucrose	5.14	6.59	12.74	5.92	10.77	16.49	20.62	14.20	28.09	52.43
Total sugars	34.94	43.72	66.63	46.54	30.93	49.30	61.94	26.33	40.05	57.31
Starch	61.07	37.07	5.18	0.43	129.91	95.78	20.79	92.98	62.52	24.32
Total carbohydrates as starch	92.37	76.59	65.52	42.44	156.46	139.93	77.45	117.75	99.29	78.18
Cellulose	8.40	9.77	11.16	11.70	13.59	14.37	14.69	11.31	10.71	10.55
Pectin	5.37	5.56	6.91	6.19	7.93	7.36	8.98	6.75	5.58	7.08
Total N as protein	8.86	7.90	7.36	7.27	12.69	11.83	10.14	11.50	10.69	10.26

of the Butternut than in those of the other varieties. The relative increases in ether extract and in total nitrogen are striking. At harvest the seeds of the Buttercup variety appeared more mature than the others, and the changes listed above are less pronounced.

In assembling the results of the 1944 samplings it became evident that extreme variations occurred in two respects: the moisture content of the fruits, and the proportion of fiber and seed in them. Individual determinations of total solids were made on each of the five fruits used for one of the Blue Hubbard samples. The results were: 15.76, 11.16, 10.84, 11.91 and 14.36 per cent. The percentages of the total solids of the whole fruit present in fiber and seeds were also extremely variable.

When the samples for this season were taken it was hoped that the results

TABLE IV

LOSSES DURING STORAGE IN PERCENTAGE OF THE AMOUNTS PRESENT AT HARVEST

	2 WEEKS	3 MONTHS	6 MONTHS
	%	%	%
Blue Hubbard			
Fresh weight	3.19	9.72	16.06
Solids	9.98	18.57	38.32
Total carbohydrates	17.08	29.07	54.05
Buttercup			
Fresh weight	9.79	22.65	.....
Solids	9.05	41.18	.....
Total carbohydrates	10.56	50.50	.....
Butternut			
Fresh weight	11.15	25.05	.....
Solids	12.39	25.18	.....
Total carbohydrates	15.68	33.60	.....

of their analysis might make possible the calculation of the material changes in flesh, fiber and seed during the storage period. The variations recorded above show that these calculations from a single season's sampling would have little, if any, value. For this reason such results for fiber and seed are not recorded. They do, however, provide some indication that there is little, if any, transfer of material from flesh to fiber and seed during storage.

A more reliable estimate of the material changes in the edible portion of the fruits was obtained as follows: Fiber and seed were omitted from the calculation. Together they comprise a comparatively small part of the whole, and as there appears to be no great change in their total weight during storage, the error introduced in this way is small. The figures in table I were used for the composition at the various storage periods. Since these are the averages for three seasons, they are more representative than the results for any one season. Since the loss in fresh weight of a large number of fruits was determined during the 1944 season, the averages of these losses, which are presented as part of table IV, were used in the calculation. The estimates obtained in this way, of the material changes in the edible portion

corresponding to a given fresh weight of fruit at harvest, are presented in table III.

In spite of the sampling difficulties, the major changes are quite evident. These include pronounced losses in solids, in starch and in total carbohydrates. The results indicate in most cases some increase in cellulose and pectin and some loss in total nitrogen. It is doubtful if the samples are sufficiently representative to provide conclusive evidence on these points. Both tables I and III emphasize the large amounts of sugars present, much of which might be lost by certain methods of processing. From the practical point of view the losses of fresh weight, of total solids and of total carbohydrates are especially important. These losses are presented in table IV in percentage of the amounts present at harvest.

Summary

A study of the composition of samples of three varieties of squash taken at harvest and after various periods of storage during three seasons leads to the following conclusions:

1. The conversion of starch to sugar is rapid and becomes practically complete on long storage.

2. The loss of carbohydrates is rapid, amounting to about one-third of the amount present at harvest after three months' storage, and one-half or more after six months' storage.

3. The proportion of glucose in the total sugars increases during storage.

4. In the Butternut, the proportion of sucrose in the total sugars is very high.

5. Since maltose was absent from the samples tested for it, it is doubtful if amylases are concerned in the transformation of starch.

6. The results indicate slight losses in protein and slight gains in cellulose and pectin. It is doubtful, however, that the sampling was adequate to provide conclusive evidence on changes in cellulose and pectin content.

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POLAR DISTRIBUTION OF RESPIRATORY RATE IN THE ONION ROOT TIP

L. Joe Berry and Mary Jane Brock

Introduction

Since the root tip of *Allium cepa* is being used more and more in physiological investigations, it was considered of value to investigate and compare the respiratory rate of different regions in this polar tissue. Lund and Kenyon (**8**) observed that the region of active mitotic division in the root tip had the greater rate of reduction of methylene blue when compared to more basal regions and that this apical region was electropositive to the higher regions of the root. They also found that there was the same polar distribution in the production of acid (CO_2) when tested with phenol red. These factors were given as evidence in support of the theory of the origin of bioelectric potentials as oxidative-reductive systems in flux equilibrium. Marsh (**9**) showed that the electrical potential difference between apical and basal contacts on the root could be depressed by KCN, and Rosene and Lund (**11**) found that the displacement of oxygen with hydrogen around the root also lowered the E.M.F. The readmission of oxygen gave a "rebound" effect in the p.d. of the root which was interpreted as resulting from a greater accumulation of oxidizable material in the apex as compared to the base. Berry and Hoyt (**1, 2**) observed that an onion root under anaerobic conditions could not be stimulated when direct or alternating current was passed through the root. Rosene (**13**) has recently reported the inhibiting effect of KCN on the rate of exudation in excised onion roots. The present paper evaluates the normal respiratory rate of three segments of the root and the problems associated with measurements of this type. There is also included a preliminary estimate of the effects of cyanide and methylene blue on oxygen consumption in these segments.

Method

The Warburg manometric method (**5**) was used for measuring the oxygen consumption of from 34–61 root segments. The dry weights varied from 3–14 mg. and the final values are expressed as cubic millimeters per root per hour. Respiration was also calculated as cu. mm. of oxygen consumed per mg. dry weight per hour (Q_{o2}) but the results were no more reproducible than those reported below. For this reason the former values alone are reported in this paper. Average values obtained in this way were checked on single roots using the Scholander microrespirometer (**15**).

The roots were grown as usual in an aerated tap-water aquarium from bulbs 10–15 mm. in diameter. The bulbs were supported on a paraffined wooden board so that the roots could grow freely into the water through holes in the board. In preliminary experiments they were permitted to grow down into open glass tubes with the bulb supported on a flanged end. More

uniform aeration of all roots was obtained when no glass tubes were used. A thermoregulator maintained the aquarium temperature at 25° ± 1° C. Roots 30–60 mm. in length were used and a total growth period of 72 ± 8 hours was employed.

Root segments were cut the desired length with small dissecting scissors having a rotating compass point soldered to one blade. For minimum injury the segments were placed immediately on moistened filter paper and transferred to the flasks by spatula. Roots from 8–15 bulbs were used, each flask having the same number of tips from each onion, with either equal lengths or 5, 10, and 15-mm. lengths used in the different flasks for one experimental run. The time required to prepare tissue limited the number of experimental flasks to three. With the temperature of the water bath at 25° C. and shaking speed at 100 oscillations per minute, the respiratory rate was constant for three hours or more and then began to decline. Only the rates of oxygen consumption measured during the initial three hours are, therefore, reported in this paper. After the experimental run, roots were dried in an electric oven at 60–70° C. and weighed to 0.5 mg.

Results

Normal respiration

Table IA summarizes the results of Warburg manometric measurements made with three different lengths of root tips, as shown in column one. The range of values calculated as cubic millimeters of oxygen consumed per root per hour is given in column four and the mean value and probable error for each segment are shown in column five. From these results it is possible to evaluate and compare the rates of oxygen consumption for the arbitrarily selected segments of root designated in column six. The values as they appear in column seven are obtained by finding the difference between the mean values in column five. The apical 5-mm. root tip has an oxygen consumption

TABLE I

Normal respiration of onion root tip

Length of root tip	Number of exps.	Number of roots per exp.	mm.³ O_2/root/hr. (range of values)	mm.³ O_2/root/hr. (mean)	Segment of root	mm.³ O_2/root/hr. (by difference)	% of apical 5 mm.
I	II	III	IV	V	VI	VII	VIII
mm.					mm.		%
A. Warburg manometers							
0– 5	31	46–61	0.783–1.189	0.997 ± .075	0– 5	(0.997)	100.0
0–10	41	36–56	1.349–2.116	1.678 ± .144	5–10	0.681	68.3
0–15	39	34–60	1.870–2.793	2.255 ± .137	10–15	0.577	57.8
B. Scholander microrespirometer							
0– 5	2	1	0.768–1.081	0.925 ± .105	0– 5	(0.925)	100.0
0–10	5	1	1.371–1.678	1.522 ± .074	5–10	0.597	64.5
0–15	7	1	1.567–2.351	1.977 ± .162	10–15	0.455	49.2

over 30 per cent. greater than the adjoining 5-mm. length and 40 per cent. greater than the segment 10–15 mm. above the tip (column right). The values thus obtained seem more reliable than actual measurements on root segments with two cut surfaces. It is reasonable to assume that the shortest segments of roots cut 5 mm. above the tip suffered proportionally greater stimulation and protoplasmic injury than those cut 10 to 15 mm. above the tip. If the increase in respiration from stimulation exceeds the decrease due to injury to the cells, the observed differences in respiratory rate of the polar segments would slightly exceed the true values. There is, however, nothing in these data that indicates the nature of the error introduced by cutting.

Results obtained with the Scholander microrespirometer are given under the same headings in Table IB. In order to test the significance of the difference between means shown in column seven, the "t" test was employed (7). This test is particularly useful for small samples and measures the probability level at which the difference in means might occur by chance. If the probability is 5 per cent., the difference is statistically significant and 1 per cent. is highly significant statistically. Both values found here gave a probability of less than 1 per cent. Oxygen consumption of individual roots was 80–86 per cent. of that obtained by the Warburg technique with roots grown on the same days; however, it is clear that the same type of polar distribution of oxygen consumption is obtained. The percentage values listed in the last column agree within 9 per cent. with those derived from Warburg measurements.

It was originally hoped that the use of a large number of segments in each manometer flask would cancel the variations in the respiratory rate of individual roots. Lack of reproducibility was observed, however, and certain environmental factors which might influence these variations were controlled. The aquarium in which the roots were grown was kept at constant temperature and the roots were permitted to develop unrestricted by tubes in an aerated aquarium in order to maintain a reasonably uniform oxygen tension. Some roots were grown in test tubes but results from these were also variable. With 10 per cent. Knop's solution substituted for tap water in the aquarium, the reproducibility of respiratory rates was not improved. It is significant, however, that in experiments set up in duplicate or triplicate on a given day with the same number of roots from a given bulb in each flask, the respiratory rates fell within the approximate limits of error of the method, less than 5 per cent., in 16 out of 20 cases. Of the four exceptions, all of which fell within 10 per cent., two were found in five tests with 5-mm. lengths of root; one in nine tests with 10-mm. lengths and one in six tests with 15-mm. lengths. These results would suggest, therefore, that the sampling for a given determination was adequate to balance out the variations in respiration found in roots of the same bulb and in roots of different bulbs subjected to approximately identical growth conditions. There are no data on the range that might be found in the oxygen consumption of roots from the same bulb but preliminary measurements show that roots reaching a

length of 30 mm. after two days' growth had 35–45 per cent. greater oxygen consumption than roots permitted to grow for four days. Since all roots at least 30 mm. long were used in each experiment and since some of these roots may have been younger than others, it may be that a comparable range of variation might be expected. ROSENE (**12**) has found that the velocity of water absorption measured as cubic millimeters of water per square millimeter of root surface per hour may show considerable difference in roots from the same bulb.

Growth conditions not controlled from day to day were room temperature and illumination. The temperature varied from approximately 14° to 21° C. and even though the bulbs were immediately above a thermostatically controlled water bath, it may be that they were influenced to some degree by this range in temperature. The roots, on the other hand, were always subject to some change in temperature during the period required to prepare them for study. There is not sufficient data available, however, to permit an accurate analysis of this effect. Since WEINTRAUB (**16**) has recently shown that light of low intensities inhibits the growth of roots, there is reason to believe that constant illumination of the growth aquarium might lead to more uniform respiration.

EFFECT OF POTASSIUM CYANIDE ON RESPIRATION

After a period of normal respiration potassium cyanide, 1/1000 M final concentration, was added to manometer flasks. All of these measurements were made in Warburg manometers, using 40 or more roots in each determination. Results are summarized in table II, where columns three and four give, respectively, the range and the mean with probable error of cubic mm. of oxygen consumed per root per hour. The average oxygen consumption of the same three 5-mm. segments was calculated by difference of the means with the results shown in column six. When the differences of the means were subjected to the "t" test they were found to be highly significant with a probability of less than 1 in 100 that the differences shown would occur by chance. When these rates are compared with the normal rate of oxygen consumption for the corresponding segment, it is seen that the first 5 mm.

TABLE II

RESPIRATION OF CYANIDE POISONED ONION ROOT TIPS

LENGTH OF ROOT TIP	NUMBER OF EXPS.	MM.³ O_2/ROOT/HR. (RANGE)	MM.³ O_2/ROOT/HR. (MEAN)	SEGMENT OF ROOT TIP	MM.³ O_2/ROOT/HR. (BY DIFFERENCE)	% OF NORMAL*
I	II	III	IV	V	VI	VII
mm.				*mm.*		%
0– 5	4	0.341–0.492	0.445 ± .042	0– 5	(0.445)	44.6
0–10	4	0.724–0.934	0.819 ± .051	5–10	0.374	55.0
0–15	3	1.234–1.416	1.318 ± .051	10–15	0.499	86.5

* This figure is the quotient of column VI with the corresponding value in Table IA column VII.

of the root tip has only about 45 per cent. of normal respiration in cyanide, whereas, the 5- to 10-mm. segment and the 10- to 15-mm. segment have 55 per cent. and 86 per cent., respectively, of normal respiration in cyanide. The metabolic activity of the cells in a polar tissue like the root seems, therefore, differentially inhibited by the action of cyanide. These results may be compared to those reported by ROSENE (13) in which Webber had found 41.55 per cent. inhibition of oxygen consumption in onion roots with 0.0025 per cent. KCN. These measurements were made with the Winkler technique over a 48-hour period at 24° C. The length of root tips employed was not specified but the value corresponds closely with our 10-mm. lengths. Webber also obtained reversible inhibition (amount not given) with 0.005 M KCN in both apical and basal segments, using the Warburg method. As COMMONER (4) has emphasized, however, the average percentage inhibition by KCN does not necessarily indicate the relative activity of cyanide-sensitive and cyanide-stable respiration. It is necessary to utilize a range of cyanide concentrations in order to find the maximum inhibition. Under these conditions, comparisons of greater validity can be made.

RESPIRATION IN CYANIDE AND METHYLENE BLUE

Since the greatest effect of cyanide was produced on the first 5-mm. segment and the minimum effect on the last 5-mm. segment, it was decided to test the combined action of cyanide and methylene blue on 5-mm. and 15-mm. lengths of root tip. The mean results, with probable errors, from seven experiments in which various sequences were followed are shown in table III.

TABLE III

ONION ROOT TIP RESPIRATION IN CYANIDE AND METHYLENE BLUE

LENGTH OF ROOT TIP	NUMBER OF EXPS.	RESPIRATION IN $\frac{\text{CN MB}}{\text{CN}}$	RESPIRATION IN $\frac{\text{CN MB}}{\text{NORMAL}}$	RESPIRATION IN $\frac{\text{CN MB}}{\text{MB}}$	RESPIRATION IN $\frac{\text{MB}}{\text{NORMAL}}$
I	II	III	IV	V	VI
mm.		%	%	%	%
0– 5	4	125.6 ± 1.68	75.5 ± 5.00	66.7 ± 1.00	109.7 ± 6.01
0–15	3	114.7 ± 3.48	77.4 ± 4.80	73.4 ± 2.60	102.1 ± 5.24

In some cases the cyanide was added previous to the addition of 0.002 per cent. methylene blue or the methylene blue was added before the cyanide. In this way it was possible to show that methylene blue increased the oxygen consumption of the first five mm. of the root tip poisoned by cyanide more than it did in basal segments as shown in column three of table III. The presence of methylene blue in cyanide-poisoned roots, for example, produced an increase of approximately 30 per cent. in respiration of the apical 5 mm., as shown in column four (when compared to column seven of table II). The less sensitive basal region showed essentially no response to the methylene blue since most of the changes seen in the 0–15-mm. segments were probably due to the apical cells. Therefore, under the conditions of these experiments

the cyanide-sensitive respiration of the apical region was approximately 50 per cent. restored to normal by the addition of the methylene blue (from 45 per cent. to 75 per cent.). Methylene blue alone produced a slight elevation above normal respiration, but this increase approaches the limit of accuracy of the Warburg technique. This and the small number of experiments render the increase of doubtful significance.

Discussion

The oxygen consumption of individual roots might be expected to show considerable variation, not only because of inherent differences, but also because of inconstant growth conditions. The fact that measurements with samples of 40 or more roots failed to compensate for these variations from day to day but were effective on a given day emphasizes the importance of the environmental factors. Since light intensity seems to be at least one factor not rigorously controlled in these experiments, it is interesting to note the rather inconsistent effects of light on respiration of colorless plant tissues reported in the literature. CANNON (**3**) compared the volume of oxygen removed from water by roots when the shoots were in direct sunlight and in dense shade. Using Salix and Helianthus, he found that in 69 per cent. of the cases less oxygen was absorbed in light. FÖCKLER (**6**), on the other hand, found that light accelerates respiration in a variety of colorless plant tissues, especially at high intensities. In no case was a retarding effect observed. The oxygen consumption of Pistia roots was reported by RANJAN (**10**) to be unaffected by light. It is thus apparent that additional work is required before any valid conclusion can be drawn regarding the rôle of light in root respiration in general and especially as it may apply to onion roots.

The effect of temperature on oxidative metabolism is well known. The roots were grown at the same temperature that was used for measuring oxygen consumption but, since the bulbs may have been subject to some cooling and since the preparation of roots for experimentation necessitated a brief exposure to room temperature, an alteration in the mean level of respiration may have occurred from day to day. The data, however, do not permit a valid conclusion on this point. The lack of uniformity encountered in these experiments is not unique since other workers using adequate samples have also observed a wide range of respiratory values. ROSS (**14**), for example, reported a range of 20 to 30 for Q_{O_2}'s of Nitella, based on one gram wet weight (corresponding to 25 mg. dry weight). This is comparable to the results observed with the onion root.

The differences in oxygen consumption which were calculated for the three 5-mm. segments of root tip and were found statistically to be highly significant might be expected from cytological evidence alone. The apical segment contains the active mitotic cells with few vacuoles and a maximum content of protoplasm while the two more basal segments have the large sap vacuoles, very little meristematic tissue and highly differentiated cells. The

mass of protoplasm becomes progressively less in going from the tip to more basal segments. Thus a greater oxygen consumption does not necessarily reveal a greater intensity of oxidative metabolism in apical cells but rather a greater quantity of metabolizing material. The differential inhibition of oxygen consumption by cyanide suggests, however, that there is a polar difference in the oxidative metabolism of the onion root. This is further borne out by the greater increase in oxygen consumption of the cyanide poisoned root when methylene blue is added to apical segments as compared to longer lengths of root. There is, therefore, direct experimental evidence for the polar difference in oxidative metabolism which has been offered as explanation for the electrical behavior of onion roots under various environmental conditions (**1, 2, 8, 9, 11**). The ultimate proof of such a polar difference must await, however, more extensive and carefully controlled investigations.

Summary

1. The average oxygen consumption in cubic millimeters per root per hour, as measured with 34–61 roots by the Warburg manometric method, is 0.997 for 5-mm., 1.678 for 10-mm., and 2.255 for 15-mm. lengths of onion root. Similar results were obtained on individual lengths of root by the Scholander microrespirometer but the average values were 80 to 85 per cent. of the above.

2. There was as much as 50 per cent. variation in day to day values in all lengths of roots studied, but the results agreed within 10 per cent. in experiments set up in duplicate or triplicate on a given day. The maintenance of certain constant growth conditions failed to eliminate the variability but room temperature and illumination were not controlled.

3. A polar difference in cyanide inhibition and in the antagonistic effect of methylene blue for cyanide poisoning was found. This was interpreted as suggesting a fundamental difference in the oxidative metabolism of the different segments of the root.

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COMPARISON OF RESPIRATION, FREE FATTY ACID FORMATION, AND CHANGES IN THE SPECTRUM OF THE SEED OIL DURING THE STORAGE OF COTTONSEED

LILLIAN KYAME AND A. M. ALTSCHUL

(WITH FIVE FIGURES)

Introduction

The phenomenon referred to as seed deterioration is a complex of a number of different biological and chemical processes. Although a number of these processes have been independently investigated, there is little available information on the relationships existing between them. For example, while it is known that increased moisture content results both in increased lipolytic and respiratory activity, it has not been established whether these two activities are independent manifestations of increased moisture content or whether they are functionally related. For this reason it was of interest to follow lipolytic activity and changes in the seed pigments in those seed samples used for the respiration measurements reported in the preceding publication of this series (**7**).

Materials and methods

The 3-pound lots of seeds that had been set aside for the respiration measurements (**7**) were used as a source of material for free fatty acid determinations. One portion of the seed oils extracted from samples periodically withdrawn from containers was analyzed for free fatty acid content, while spectrophotometric measurements were made on another portion. The free fatty acid analyses were carried out according to the method previously described by KARON and ALTSCHUL (**6**), and the spectrophotometric measurements as described by ALTSCHUL and coworkers (**1**).

In all that follows, moisture content is expressed on the basis of the total wet weight of the seed, and the amount of free fatty acids in terms of percentage of oleic acid. Spectrophotometric measurements were conducted in a Coleman double monochromator spectrophotometer with an absorption cell thickness of 13 mm.; absorption data refer to a solution containing 1 ml. of oil in a total volume of 50 ml., carbon tetrachloride being used as the solvent.

Free fatty acid formation

In a previous publication (**6**), it was shown that the rate of lipolysis in cottonseed can be evaluated by the use of the autocatalytic-type equation:

$$dF/dt = k(F)(100 - F) \qquad (1)$$

where F = the percentage of free fatty acids formed,
$100 - F$ = the percentage of residual unhydrolyzed fat,
t = the number of days of storage.

Thus the numerical value of the rate constant, k, in equation (1) quantitatively characterizes the course of free fatty acid formation in cottonseed.

From the data obtained by periodic free fatty acid analyses upon 18 lots of seed, values of the rate constant, k, were determined (table I). It will be noted that there is no evaluation of k for three of the seed lots. The rate of formation of free fatty acids in lot 205a was too low to be accurately measured. The seeds in lots 204a and 204b gave hydrolysis patterns which

TABLE I

THE EFFECT OF MOISTURE ON THE RATE CONSTANT, K, FOR THE FORMATION OF FREE FATTY ACIDS IN STORED COTTONSEED

SAMPLE	VARIETY	DATE OF HARVEST, 1942	MOISTURE CONTENT	MOISTURE TREATMENT	K
			%		*reciprocal of days*
104b	Delfos 3506	Aug. 29	10.0	Lowered	1.7×10^{-5}
105b	"	Oct. 3	10.6	None	2.2 "
104a	"	Aug. 29	12.0	None	2.0 "
105a	"	Oct. 3	13.1	Raised	5.0 "
105f	"	"	14.2	Raised	8.7 "
105f′	"	"	14.9	Raised	13.6 "
105e	"	"	16.8	Raised	17.6 "
205a	Coker's 200, strain 1	Sept. 22	12.5	Raised	.....
204b	"	"	12.5	Lowered	.....
204a	"	"	13.7	None	.....
206a	"	Oct. 22	14.7	Raised	6.0 "
205e	"	Sept. 22	15.9	Raised	13.6 "
305b	Oklahoma Triumph, 0–52–12	Oct. 1	10.6	None	1.4 "
306b	"	Oct. 28	10.6	None	2.4 "
307a	"	Nov. 28	12.9	Raised	3.2 "
306a	"	Oct. 28	13.1	Raised	2.6 "
304a	"	Sept. 22	13.3	Raised	3.6 "
308a	"	Dec. 28	15.7	None	12.9 "

were fundamentally different from those of the other seed samples investigated.

The values of k are plotted as a function of moisture content in figure 1. The experimental points for the samples in the 100 series all fall on one curve, whereas the points for the samples in the 200 and 300 series fall on a second curve which is lower than the first. In this respect the results of the lipolysis rate determinations resemble very closely those of the respiration measurements on the same seed sample (7), since in both instances a higher level of activity at comparable moisture contents was exhibited by the seeds in the 100 series.

The curves in figure 1 lend themselves to further analysis by the use of the following autocatalytic-type equation:

$$dk/d(H_2O) = k'k(A - k) \qquad (2)$$

where k = lipolysis rate constant,
(H_2O) = percentage moisture in the seed,
k' = constant relating moisture content to lipolysis rate constant
A = arbitrary constant.

Upon integration, equation (2) becomes

$$\log k/A - k = A\,k'\ (H_2O)/2.3 + \text{constant}. \qquad (3)$$

If the equation is applicable to the curves in figure 1, there should exist a value of A for which a plot of $\log k/A - k$ against (H_2O) will give a straight

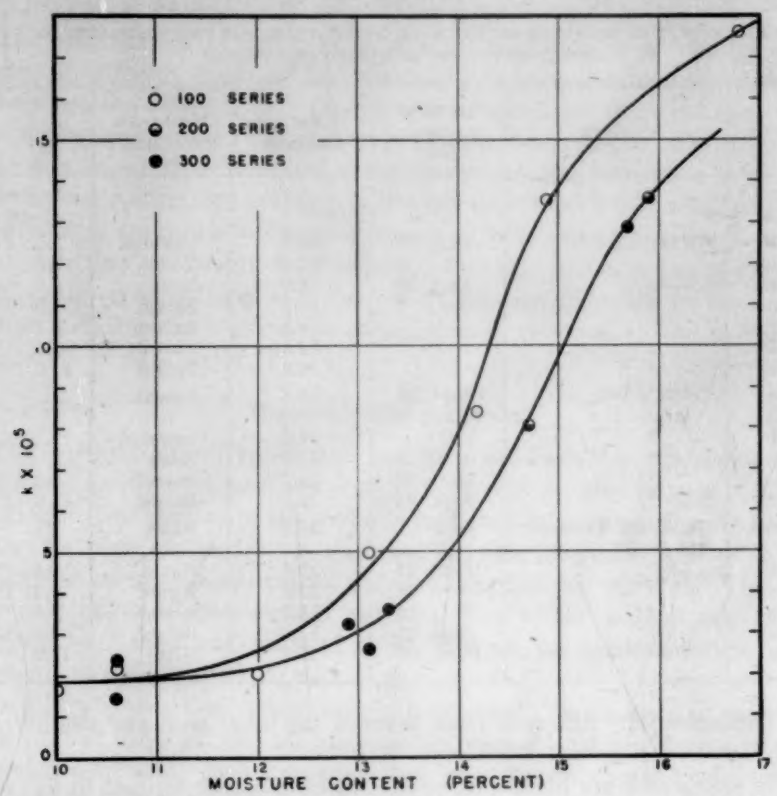

FIG. 1. The effect of moisture content on the lipolysis rate constant of cottonseed.

line with a slope equal to $A\,k'/2.3$. When a value of 50×10^{-5} was chosen for A, it was found that straight lines could be obtained to represent in a linear manner the effect of moisture on a function of the lipolysis rate constant. As is shown in figure 2, the data for the seeds in the 100 series fall on one straight line in the range of 11–16 per cent. moisture and the data for the seeds in the other two series fall on a second straight line. In order to compare the properties of the present seed samples with those used in a previous investigation (**6**), the rate constants for these seed samples were

treated in the same manner and here again a straight line was obtained[1] (curve 1, figure 2). The slopes of the lines in figure 2 are 0.27 per one per cent. moisture for the seeds in the 200–300 series, 0.33 for those in the 100 series, and 0.43 for the "Delfos" (6) seeds harvested in 1941.

It is interesting to note that the methods of analysis used to express respiration intensity and rate of hydrolysis as linear functions of the mois-

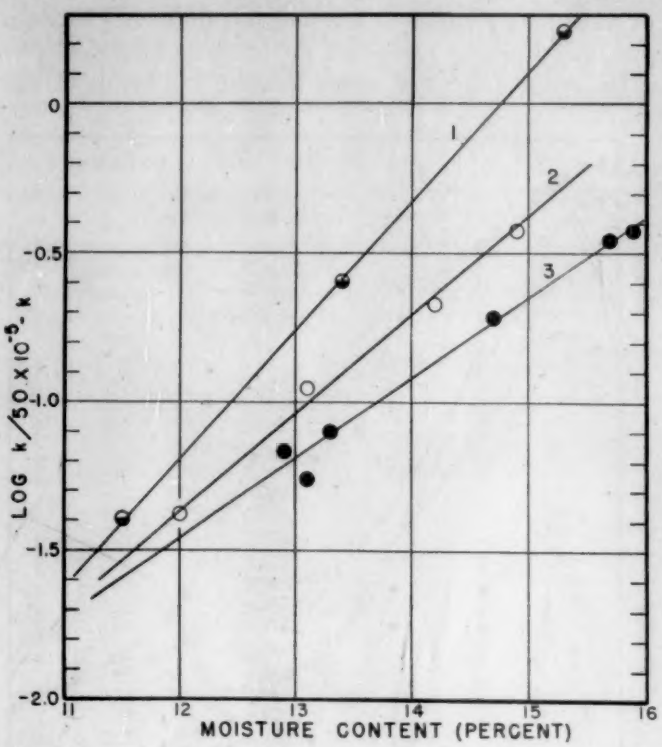

FIG. 2. The effect of moisture content of cottonseed on a function of the lipolysis rate constant.

1. "Delfos" variety of cottonseed (6), harvested in 1941.
2. 100 series of seeds, "Delfos" variety harvested in 1942.
3. 200 and 300 series of seeds, "Coker's" and "Oklahoma Triumph" varieties, harvested in 1942.

ture content indicate different types of relationship between seeds in the 100 series and the 200 and 300 series. From the respiration data it was found that the seeds in the 200 and 300 series exhibited the same sensitivity to increase in moisture as did the seeds in the 100 series, manifested by the fact that the slopes of the two lines representing these series were the same (7). The two series differed, however, in that the 100 series respired at a

[1] In order to compare previous results (6) with those reported in this investigation, it was necessary to convert the values for the percentage moisture content from the dry basis used previously to a wet basis as reported in this publication.

higher level of intensity. The hydrolysis data, on the other hand, revealed that the seeds of the 100 series exhibited a higher level of lipolysis and also displayed a higher sensitivity to increased moisture than those in the 200 and 300 series. In both lots practically the same rate of lipolysis occurred at a moisture content of 11 per cent., but great differences were observed at higher moisture values.

It is not immediately apparent whether this difference between the rate of respiration and lipolysis represents an intrinsic biological phenomenon or whether the relationship that seems to exist is a result of the methods

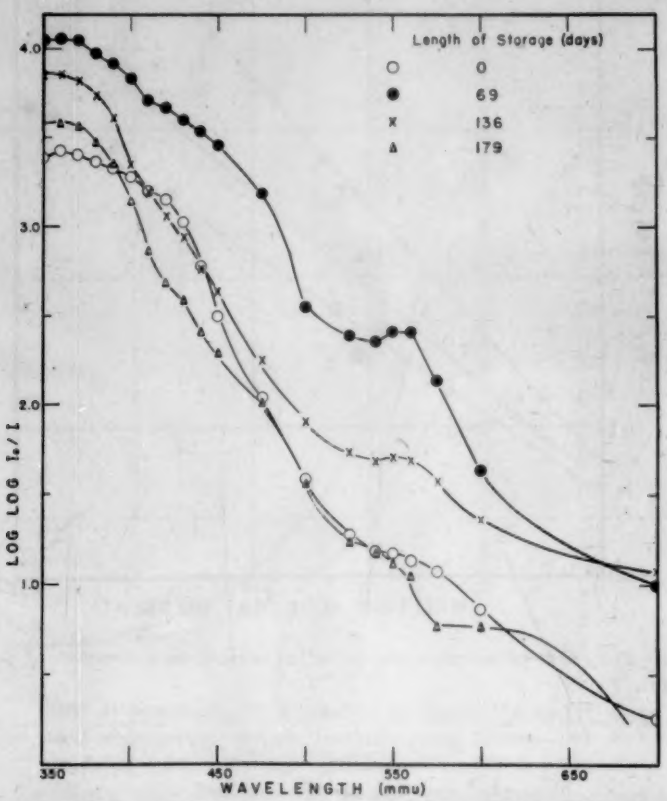

FIG. 3. The variation in the absorption spectrum of extracted oils from sample 205e during storage.

of mathematical analysis used. For all practical purposes the respiratory intensity, defined as the average respiration rate of the seed, is independent of the character of the respiratory pattern. On the other hand, the hydrolysis-rate constant, k, is determined by the rate of increase in free fatty acids in the seeds as a function of the length of storage. It is quite possible, therefore, that two such different constants would not respond in the same manner to mathematical analysis. Unfortunately, because of the irregular nature of the respiratory patterns, it is not possible to use any but an aver-

aging process to arrive at a quantitative value for respiratory intensity. On this basis there cannot be obtained a mathematical measure of the sensitivity of respiratory intensity to moisture which will be wholly comparable to that for lipolysis.

Absorption spectra of solvent-extracted oils

When cottonseed flakes or meats are extracted with fat solvents, there are extracted along with the oil a considerable quantity of pigments whose nature has been the subject of many investigations. As a result of such investigations, a new class of pigments peculiar to cottonseed has been

TABLE II

THE RELATIVE LIGHT ABSORPTION COEFFICIENT AT 560 mμ OF THE OILS FROM THE COTTONSEED SAMPLES IN THE 100 SERIES

SAMPLE	LENGTH OF STORAGE	LOG I_o/I	SAMPLE	LENGTH OF STORAGE	LOG I_o/I
	days			*days*	
104b	0	0.012	104a	0	0.021
	87	0.010		52	0.012
	232	0.019		115	0.007
	367	0.032		218	0.014
				367	0.037
105a	0	0.005	105b	0	0.011
	27	0.009		71	0.010
	122	0.011		160	0.008
	328	0.021		342	0.026
105f	0	0.013	105f′	29	0.018
	66	0.015		120	0.017
	120	0.016		156	0.011
	337	0.016		337	0.028
105e	27	0.036			
	62	0.032			
	111	0.012			
	147	0.020			
	223	0.093			

disclosed. Of these, gossypol, a yellow pigment, is the most abundant and the best known. BOATNER *et al.* (**3**, **4**) have shown, however, that at least four pigments besides gossypol exist in cottonseed and that some of these, at least, are interrelated.

Much of the pigmented material in the cottonseed is localized in structures known as pigment glands distributed throughout the embryo (**8**). All of the pigments so far isolated have been found in these glands, from which they can be readily extracted by use of suitable solvents (**5**). There are solvents, however, that are incapable of breaking up the glandular structure. These solvents are able to extract only those pigments that occur outside the glands or are contained in the small number of ruptured glands present in cottonseed flakes or ground cottonseed. An example of solvents of the latter type is Skellysolve F (low-boiling petroleum ether).

In the experiments reported above, petroleum ether was used to extract

TABLE III

THE RELATIVE LIGHT ABSORPTION COEFFICIENT AT 560 mμ OF THE OILS FROM THE COTTONSEED SAMPLES IN THE 200 SERIES

SAMPLE	LENGTH OF STORAGE	LOG I_o/I	SAMPLE	LENGTH OF STORAGE	LOG I_o/I
	days			*days*	
204a	0	0.030	204b	0	0.041
	69	0.036		31	0.031
	142	0.051		67	0.033
	212	0.071		140	0.048
				210	0.074
205a	0	0.044	206a	0	0.017
	29	0.025		56	0.013
	179	0.025		109	0.013
	338	0.064		244	0.071
				316	0.043
205e	0	0.014			
	69	0.258			
	136	0.048			
	179	0.011			

the seed oil which was then analyzed for free fatty acids. On aliquots of the same extracted oil, periodic spectroscopic determinations were also made. As an example of the type of results obtained in the spectroscopic analyses, the data for sample 205e are given in figure 3. It will be noted that as storage progresses there is a change in the spectra of the oils. At 560 mμ, the point of maximum light absorption of the pigment gossypurpurin (**3**), there is first an increase in absorption, which is followed by a decrease as the storage is continued. The light-absorption value after 179 days of storage is very close to the original value. A similar pattern of

TABLE IV

THE RELATIVE LIGHT ABSORPTION COEFFICIENT AT 560 mμ OF THE OILS FROM THE COTTONSEED SAMPLES IN THE 300 SERIES

SAMPLE	LENGTH OF STORAGE	LOG I_o/I	SAMPLE	LENGTH OF STORAGE	LOG I_o/I
	days			*days*	
304a	0	0.007	305b	0	0.020
	32	0.022		71	0.019
	91	0.026		267	0.070
	146	0.038		339	0.053
	225	0.100			
	327	0.105			
306a	0	0.032	306b	0	0.018
	56	0.030		68	0.043
	110	0.040		323	0.068
	212	0.084			
	317	0.058			
307a	0	0.022	308a	0	0.021
	64	0.023		55	0.072
	144	0.043		133	0.088
	282	0.079		237	0.012

change is observed in the absorption at 360 mμ, a point of maximum light absorption of gossypol (3). Again there is an increase in the light absorption during the initial stages of storage followed by a decrease as storage is continued.

The effect of storage on the light absorption of the oil at 560 mμ is given in tables II, III, and IV. Inasmuch as it has already been shown that the 200 and 300 series of seeds behave similarly with respect to both their respiration behavior and lipolysis rates, it was of interest to examine the experimental data to determine whether there might be a similar correlation between the

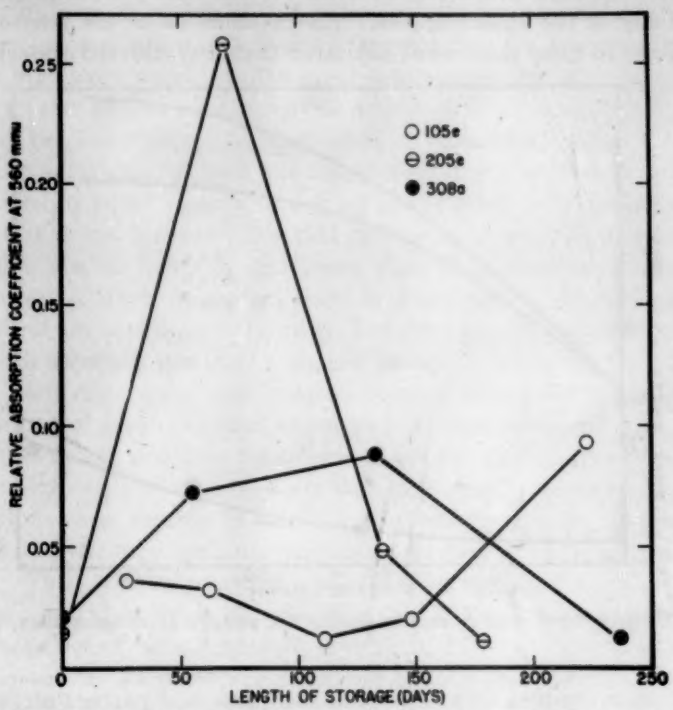

FIG. 4. The effect of storage on the relative light absorption coefficient at 550 mμ in the case of high-moisture seeds.

changes in the light absorption of the oils and other seed characteristics. Accordingly, the relative absorption coefficient at 550 mμ for the sample having the highest moisture content in each series was plotted against the days of storage (fig. 4). It is significant to note that the patterns of change in the 200 and 300 series are similar in that they both exhibit a maximum during the storage period. On the other hand, the pattern in the 100 series is reversed; *i.e.*, there is a minimum in absorption during the same storage interval.

As yet too little is known concerning the chemistry and biochemistry of cottonseed pigments to permit one to draw any significant conclusions from the above-mentioned data. Inasmuch as Skellysolve F does not rupture the pigment glands, the observed changes in light absorption may be due

both to changes in the relative pigment concentrations in the seeds and glands and to changes in the amount of Skellysolve F–soluble pigmented material which occurs outside of the glands. As soon as more information concerning cottonseed pigments is available, it should be possible to interpret data of this type with more certainty and to correlate them with other seed characteristics.

Immature seed

In the preceding publication (7) it was indicated that the samples of immature seed, 204a and 204b, exhibited a much higher level of respiration than did any of the other samples. An examination of the course of lipolytic activity in these same seeds indicated that they differed materially also

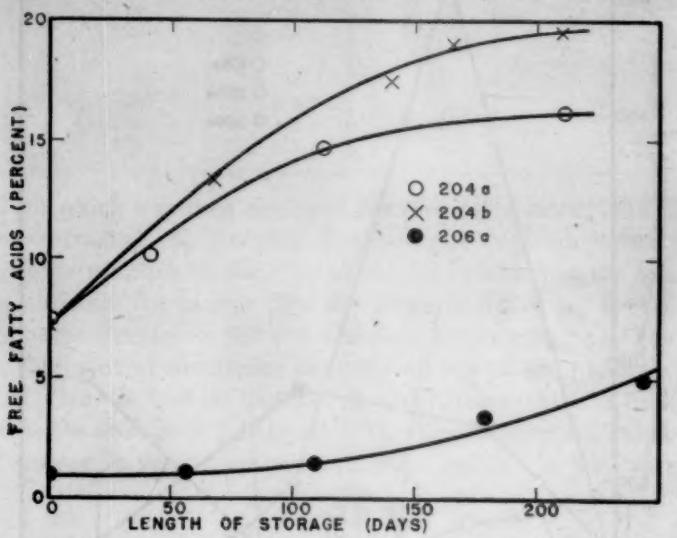

FIG. 5. Free fatty acid formation during the storage of samples 204a, 204b, and 206a.

from the other samples with respect to their rate and pattern of formation of free fatty acids. Whereas all of the other seed samples exhibited a normal accelerating rate of lipolysis as storage continued (6), the immature seeds exhibited the highest rate of lipolysis at the beginning of the storage period. As is shown in figure 5, where the curves for the rate of development of free fatty acids in samples 204a and 204b are compared to a curve for normal seeds of about the same moisture content, the rate of formation of free fatty acids in the immature seeds at the end of 200 days' storage was practically zero. On the other hand, the normal seeds were just beginning to display an appreciable rate of lipolysis at that time.

Discussion

There would appear to be two alternative explanations of the parallel relationship which exists between the vigor as reflected by lipolytic rate and the vigor as reflected by respiratory rate of the three series of seeds investigated: (1) the two processes depend on the same biological systems, or (2)

they reflect a general condition in the seeds whereby all types of enzymatic processes are maintained at a similar level of activity. The former explanation, *i.e.*, that lipolysis and respiration are functionally related, is probably not correct, in view of the differential effect of inhibitors on these two processes as shown in another publication (**2**).

It seems evident that the intensity of respiration in seeds is dependent on the amounts of the respiratory and associated enzyme systems that are present in an active state and on the rate of release or production of additional active enzymes (**7**). Similarly, the rate of lipolysis is determined by the initial concentration of active lipases and by the ability of the cell systems to release additional lipolytic enzymes as the storage progresses. The fact that seeds which exhibit equal respiratory activities (the 200 and 300 series) also exhibit equal lipolytic activities is a strong indication that seeds may be characterized by their state of "biological vigor." At least for the two variables, lipolysis and respiration, vigor with respect to one is accompanied by equal vigor with respect to the other. On the basis of this reasoning it is not inconceivable that the seeds in the 200 and 300 series would show similar behavior and lower vigor than those in the 100 series with respect to other properties such as germination, strength, and rate of growth of the seedlings. There is, however, no evidence in the present work which substantiates such a generalization.

That both respiration and lipolysis equally reflect the vigor of cotton seeds is a fact of great practical importance in commercial storage. On the basis of this fact it would be reasonable to predict that seeds whose respiration intensity is high—that is, seeds that heat readily when stored in bulk would, even when heating is controlled by air circulation, undergo more intensive lipolysis than seeds of equal moisture content but lower respiratory activity. Thus, the measurement of the heat resulting from respiration should be a useful criterion in predicting the storage behavior of seeds.

The behavior of immature seeds is entirely different from that exhibited by mature seeds. In immature seeds, the enzyme systems have not reached the state of dormancy which characterizes them in mature seeds. For this reason immature seeds are more sensitive to moisture and respire at a higher intensity than do mature seeds. The course of lipolysis in immature seeds indicates that the hydrolytic activity is due exclusively to enzymes already present in the active state and that no production of additional active enzymes takes place during the course of storage. Actually, it would seem that the enzyme systems responsible for free fatty acid formation are rendered less and less active as the storage of immature seeds progresses. In effect, then, the maturation process which is interrupted by early harvesting of the cotton, is continued during storage.

Summary

1. Samples of cottonseed that were used in the respiration investigations reported in a previous paper (**7**) were analyzed for free fatty acid content, and the lipolysis-rate constant, k, was determined for each of the samples.

2. When the lipolysis constants were plotted as a function of moisture content, it was found that the constants for the "Delfos" seed all fell on a smooth curve and that constants for the "Coker's" and "Oklahoma Triumph" varieties fell on a second smooth curve which was lower than the "Delfos" curve.

3. It was found that the lipolysis constant can be converted into a linear function of the moisture content of the seed by the use of the differential equation:

$$dk/d(H_2O) = k' k (A - k)$$

where k = hydrolysis rate constant,
(H_2O) = percentage moisture in the seed,
k' = constant relating moisture content to lipolysis rate constant
A = arbitrary constant.

When A is given the value of 50×10^{-5}, the log of $k/A - k$ becomes a linear function of the moisture content.

4. The slopes of the lines denoting lipolysis as a linear function of moisture content were found to be 0.27 unit per one per cent. moisture for the "Coker's" and "Oklahoma Triumph" seeds and 0.33 unit for the "Delfos" seeds.

5. A variation of the spectrum of solvent-extracted oils during the storage of cottonseed was found to exist. The highest moisture samples of the "Coker's" and "Oklahoma Triumph" varieties showed a common pattern which was in turn different from that exhibited by "Delfos" variety.

6. The pattern of lipolysis for immature seeds was shown to be different from that for normal cottonseed in that the rate of formation of free fatty acids decreased with length of storage.

7. The "Delfos" seeds exhibited more vigor both with respect to lipolysis and respiration than did the seeds in the "Coker's" and "Oklahoma Triumph" varieties.

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OSMOTIC PRESSURE DETERMINATIONS WITH ISOLATED PROTOPLASMIC PROTEINS

J. LEVITT

(WITH SIX FIGURES)

LOEB (**9**), KUNTZ *et al.* (**6**), SCATCHARD *et al.* (**11**), and others have made use of osmotic pressure measurements to calculate the molecular weights of pure proteins such as gelatin, crystallized enzymes, and crystallized serum albumen. Protoplasmic proteins have not been investigated. Yet it would be of some interest to know the average molecular weight of a plant's protoplasmic proteins. Such information is essential if, for instance, one is to test theories of protein splitting at low temperature. Curves obtained by plotting pressure against concentration also yield information about the relative hydration ("bound water") of the protein, for as the concentration increases, a larger and larger portion of the pressure is due to the hydration rather than the number of molecules present. The adaptation of a plant to unfavorable environmental conditions is frequently ascribed to increased hydration of proteins (**4**). Evidence for such theories has heretofore been indirect; *e.g.*, from measurements of protoplasmic viscosity or from the properties of plant juices which come mainly from the vacuole rather than the protoplasm (**7**).

Methods

ISOLATION OF PROTEINS

In the following investigation, potato tubers (Russet variety) were used, partly because of the constant availability of material and partly because no proteins other than protoplasmic are to be found (except some crystals in cells immediately below the periderm which were removed with the peel).

One reason why protoplasmic proteins have not been investigated is the difficulty in obtaining them in the undenatured state. Preliminary experiments were therefore made with the filtrate from Waring blender mash. The proteins were precipitated from the filtrate with $(NH_4)_2SO_4$, filtered off, then dialyzed. The importance of pH soon became evident. If no precautions were taken to regulate the pH, very little protein was obtained. If enough K_2HPO_4 solution was pipetted into the blender (before mashing) to obtain a pH of about 7.0 in the filtrate, a considerable amount of protein was obtained in the soluble state. If, then, enough NH_4OH was added to neutralize the $(NH_4)_2SO_4$ before precipitating the proteins, 80–90 per cent. of the extracted protein remained in the soluble state. Solubility was determined by centrifuging for 5 minutes at 600 g. then weighing the precipitated and unprecipitated proteins.

There are some disadvantages in the use of the Waring blender. Large quantities of material are needed, a relatively small fraction of the cells is broken open, only succulent tissues can be mashed, and denaturation of pro-

teins may possibly occur due to the frothing and agitation. Consequently this method was discarded in favor of freeze-drying the tissues followed by grinding in a miniature Wiley mill and extraction with K_2HPO_4.

Since FLOSDORF and MUDD'S (**2**) original work, many modifications of the "lyophile apparatus" have been described (**1, 5, 8, 10, 12**). The commercial "cryoscopic dryers" avoid the need of dry ice (except for preliminary freezing) by using a desiccant that can be regenerated.

All the above models were designed primarily for drying liquids. Even those that use the wider-necked flasks are not easy to fill and empty when pieces of tissue are used. Furthermore, the relatively close packing of the tissues does not favor rapid drying. After using a "lyophile apparatus" for some time, it was discarded for the following simpler, more efficient, less time-consuming method.

The tubers were cut into slices 2–3 mm. thick, wiped dry, then frozen in dry ice. When completely frozen, they were arranged in layers in a vacuum desiccator between alternating layers of Al_2O_3. The desiccator was evacuated to a pressure well below 1 mm. then transferred to a cold room at 0–5° C. After 24 hours, the potato slices were dry and pith-like in texture. If the slices are permitted to melt before drying is complete (due to insufficient evacuation or Al_2O_3), the melted part when dry is horny hard. Consequently, there is no difficulty in determining whether the tissues were really dried in the frozen state. The method is uniformly successful if a good high-vacuum grease is used and if there is at least 10 times as much Al_2O_3 as the water content of the tissues. Ringing the desiccator joint with Apiezon is a useful precaution to insure maintenance of the vacuum. One desiccator can be used to dry as much as 400 grams of potatoes, containing about 300 grams of water. It is, of course, not necessary to bury the slices in the desiccant, but this is a convenient and simple method where large quantities are to be dried.

The dried tissues were ground in a Wiley mill until they passed the finest screen (60 mesh). Ten grams were then extracted with 35 ml. of a solution of 0.2 M K_2HPO_4 containing 0.01 M KCN. The KCN was included to inhibit enzyme action. It at least prevented the production of the dark oxidation product which was otherwise produced and adsorbed by the proteins. Precipitation was obtained by adding 15 gm. $(NH_4)_2SO_4$ and 2 ml. of 0.75 N NH_4OH to 30 ml. of extract. The pH of the solutions was always between 7.0 and 7.6. The precipitated proteins were filtered off with hard filter paper, from which they were easily scraped without removing the cellulose fibers from the paper. The quantity of protein obtained was 1.3 to 2.0 per cent. of the dry matter of the tubers. Further extraction with alkali failed to yield any more proteins precipitable with $(NH_4)_2SO_4$.

MEASUREMENT OF OSMOTIC PRESSURE

Though indirect methods can be used, and are often preferable when working with substances of low molecular weight, in the case of proteins the

direct method is simpler and more accurate. Semipermeable membranes are easily constructed from collodion. Since the protein comprises a small percentage of the dry matter, and since the osmotic pressure of protein solutions is low enough to require high concentrations, a micromethod must be used. Furthermore, the crude protein obtained contains not only the structural proteins but all of the enzymes as well; consequently precautions must be taken to reduce chemical changes to a minimum. This requires maximum speed of dialysis and low temperature. Fortunately, the use of a micromethod results in a high speed of dialysis due to the large specific surface and the small amount of substance that has to dialyze away. In an attempt to speed up the process still further, electrodialysis was tried, but the proteins were at least partially denatured by the weakest current that was capable of reducing the time of dialysis.

The microcups were made as follows: glass rods 3 mm. in diameter were dipped into a collodion solution (10 gm. parlodion dissolved in 50 ml. absolute ethyl alcohol, 50 ml. ether, 1 ml. glacial acetic acid). After draining for about 45 seconds they were allowed to dry for 10 minutes in the inverted position. They were then immersed in 95 per cent. alcohol, an incision was made around the rod 6 cm. from the base, the cups slipped off, and stored in the 95 per cent. alcohol overnight or longer.

The manometers were constructed from 1-mm. bore capillary tubing (fig. 1). Pressure was regulated by means of a screw clamp attached to the rubber tubing at the base of the manometer. A small bulb was blown in the short arm of the manometer just opposite the zero mark on the long, graduated arm. This served the dual purpose of having a relatively large volume of air between the mercury and the microcup, and of keeping the level of the mercury in the short arm practically stationary.

The tip of the manometer was drawn out to a diameter of about 3 mm. While in the 95 per cent. alcohol, the microcups were plastic. Consequently, they could easily be forced onto the tip of the manometer, after which they were immersed in water. This hardened them in the molded shape. The capacity of a cup (up to the fitted neck) was about 0.2 ml. They were now ready to be filled with the protein precipitate, following which each cup was attached to a manometer and a rubber sleeve slipped over the connection. If the cups had been firmly forced onto the manometer, they would now stand at least three atmospheres of pressure (higher pressures have not been tried) for hours without rupturing, coming loose, or even stretching.

The whole apparatus was set up at + 2° C. Enough protein was obtained from 10 gm. of dry matter to set up five or six manometers simultaneously. The precipitate was transferred to the cups by means of a glass rod 1–2 mm. in diameter. Tight packing was achieved by centrifuging for one-half minute. Dialysis of the $(NH_4)_2SO_4$ was quite rapid. Tests of the dialysate with $BaCl_2$, as well as measurements of the electric current conducted by the protein, revealed that all the $(NH_4)_2SO_4$ (as far as can be detected by these methods) had dialysed away within 12 hours even when highly

concentrated protein gels were used. To be certain that equilibrium had been reached, the standard procedure adopted was to allow 24 hours, during which three changes of water were used (tall jars were found to be as satisfactory as running water since the dense $(NH_4)_2SO_4$ solution sank to the bottom leaving almost pure water in contact with the membranes). Of course, it was not necessary to test for the end point since this was revealed

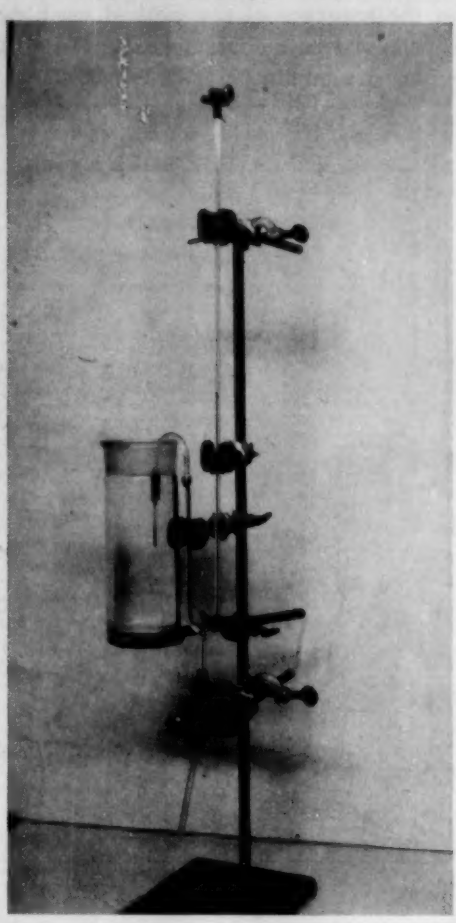

FIG. 1. Manometer with microcup attached for dialysis and osmotic pressure measurement.

by the pressure reading on the manometer. The initial pressures were large, due to the $(NH_4)_2SO_4$, consequently the manometers were closed by screw clamps for the first few hours to prevent the mercury from going over the top and the protein from taking up too much water. The pressure then dropped, finally reaching a steady rate of drop of 1–5 mm. per hour. A perfectly constant pressure was never attained due to the slow solution and diffusion of the compressed air above the protein. But the change was so

gradual as to produce no significant error. At the end of 24 hours, readings were made, the proteins transferred to weighing bottles (by squeezing them out of the cups like toothpaste from a tube) and dried at 100–110° C. to determine the moisture content.

Results

In order to test the accuracy of the method used, determinations were made with a pure protein whose characteristics are already well known—crystallized bovine plasma albumin (11). Figure 2 shows the curve obtained

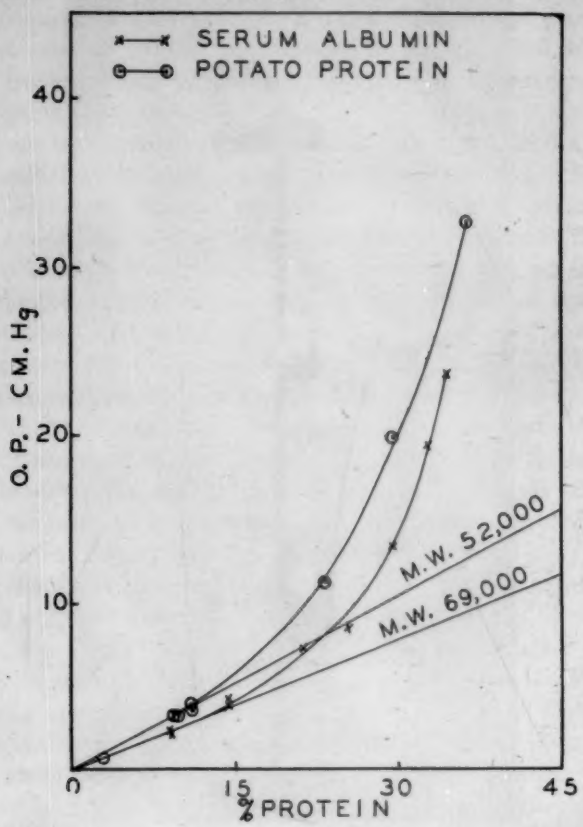

FIG. 2. Osmotic pressure curves for crystallized bovine serum albumin and protein from potato tubers.

with this protein. From the formula $M = \frac{gRT}{PV}$, the molecular weight can be calculated, using the lowest values, which are on the extrapolated, straight line portion of the curve. The value for V (the volume of solution containing g grams protein) was calculated by adding the weight of the water to half the weight of the dissolved protein. That this yields a sufficiently reliable result was indicated by dissolving 1 gram albumin in 9 ml. water. The volume of the solution was 9.6 ml.

From the curve in figure 2, the molecular weight of the albumin was calculated to be 69,000—the same value as obtained by SCATCHARD *et al.* (11). The average molecular weight of the proteins obtained from a potato tuber was similarly calculated to be 52,000 (fig. 2). The tuber had been kept at 0° C. for some time, and was tested in February. Since the potato proteins consist of a mixture of a large number of different proteins, this average molecular weight cannot be constant, such as the value for the single, pure bovine plasma protein.

The purpose of the next experiment was to determine whether or not the osmotic pressure curve is the same for proteins from growing tissues as for

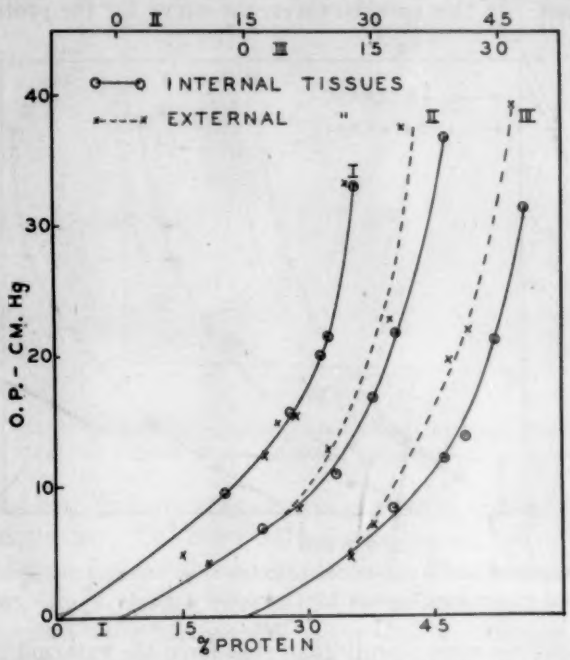

FIG. 3. Osmotic pressure curves for proteins from internal and external tissues of potato. Tubers were peeled then allowed to form a new periderm at 25° C. for 32 hours (I), 2 days (II), and 7 days (III), respectively.

proteins from non-growing tissues. To obtain tissues in the growing state, tubers from the cold chamber were peeled and placed in a large Petri plate over a layer of water at about 25° C. After 3–6 days, microscopic observation revealed the presence of a phellogen layer several cell layers below the surface. The tubers were again peeled, both the new peel (which we will call external tissues) and the remainder of the tuber (internal tissues) were freeze-dried, ground and extracted as described above. Osmotic pressure curves for both sets of tissues are shown in figure 3.

With the two longer exposures to room temperature, the curves diverge markedly at the higher concentrations and converge at the lower. Obviously, there is no appreciable difference in the average molecular weight of

the proteins from the growing and dormant tissues. The divergence at the higher concentrations indicates a higher hydration of the proteins from the growing tissues.

Figure 4 shows the results for control (unpeeled) tubers which were also kept at 25° C. for 6 days. The relative humidity, however, was that of the room in order to prevent any sprouting. At the end of the period, the tubers were peeled and the peel was discarded; the "external tissues" were then removed and compared with the "internal tissues" as in the case of the tubers that were permitted to form phellogen. In two of the three experiments, there is again no difference in average molecular weight of the proteins from the two regions. In this case, however, the curve for the proteins from the

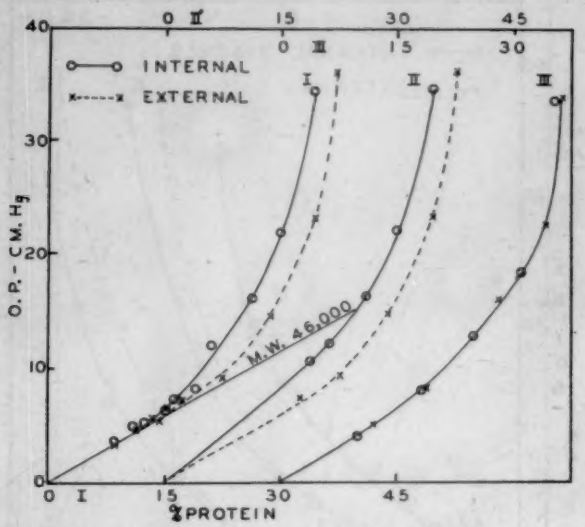

FIG. 4. Osmotic pressure curves for proteins from internal and external tissues of potato. Unpeeled tubers were kept at 25° C. for 6–7 days.

internal tissues rose more steeply than that from the external tissues in two of the three experiments. In the third experiment there was no difference.

Since the controls were not kept at the same relative humidity as the tubers that formed phellogen, in the next experiment the effect of this factor was investigated. Tubers were half peeled and then kept in the nearly saturated atmosphere at 25° C. Only the external tissues from the two regions were compared. Figure 5 shows that there is no appreciable difference between the osmotic pressure curves for the proteins from the peridermed and non-peridermed external tissues. It was noticed, however, that the unpeeled part of the tubers had begun to sprout. Consequently, neither region could be considered dormant.

These results pointed to the desirability of following the changes in the same tissues. Consequently, in the next tests, both external and internal tissues were investigated before and after phellogen formation. Tubers from the cold chamber were cut in half across the short axis. One apical

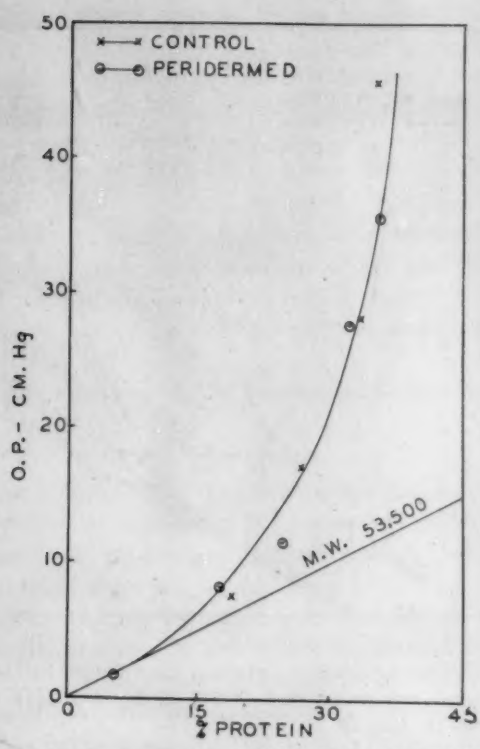

FIG. 5. Osmotic pressure curves for proteins from external tissues of half peeled tubers kept at 25° C. for 6 days. The peeled half formed periderm during this time.

and one basal half from different tubers were then separated into external and internal tissues. The other halves were peeled and allowed to form phellogen. Figure 6 shows the results obtained. They agree with the results reported above, in showing a steeper rise for proteins from external tissues in the peridermed tuber halves. The curves for the proteins from the peridermed external tissue are also steeper than those for the proteins from the control external tissues. This difference is solely due to a difference in average molecular weight. Thus the ratios of the pressures in the first set of curves at 9 and at 33.7 per cent. (the two extremes for the phellogened exter-

TABLE I

CHANGE IN QUANTITY OF PROTEINS ON TRANSFER OF TUBERS FROM 0° C. TO 25° C. FOR 6–7 DAYS (MGM. PROTEIN PER GM. DRY MATTER)

TREATMENT	INTERNAL TISSUES		EXTERNAL TISSUES	
	0° C.	25° C.	0° C.	25° C.
	mgm.	*mgm.*	*mgm.*	*mgm.*
Peeled and allowed to form new periderm	14.1	19.8	17.5	19.2
	13.0	16.8	14.6	18.1
Unpeeled	15.2	16.0	16.6	21.8

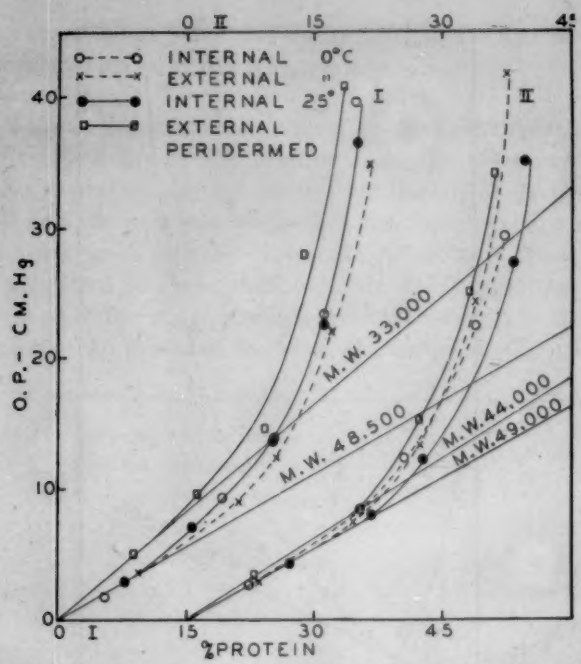

FIG. 6. Osmotic pressure curves for proteins from potato halves at 0° C. and corresponding halves peeled and transferred to 25° C. for 6–7 days to form periderm. Series I, May 1; Series II, May 19.

nal tissues) are identical (1.7). The difference between the proteins from the internal and external tissues of the controls is solely due to a difference in hydration, since the molecular weights are identical.

Any interpretation of these changes in the average properties of the proteins may follow one of two paths. They may be due to a change in the proteins *in situ* or to a transfer of proteins from one region to the other. In all the above tests it was always evident that there were more proteins obtained from the tissues kept at room temperature than from those taken directly from the cold chamber. Quantitative determinations were therefore made (table I).

TABLE II

AVERAGE MOLECULAR WEIGHT IN GRAMS OF PROTEINS FROM POTATO TUBERS

DATE	INTERNAL TISSUES		EXTERNAL TISSUES	
	0° C.	6–7 DAYS AT 25° C.	0° C.	6–7 DAYS AT 25° C.
March 11	46,000	.....	46,000	.....
April 26	.....	.....	.....	53,500
May 1	48,500	48,500	48,500	33,000 (new periderm)
May 20	49,500	49,500	49,500	44,000 (new periderm)
May 11	45,000	45,000	45,000	45,000
May 30	39,000	39,000	39,000	39,000

In both the external and internal tissues a marked increase in proteins occurred. It is quite possible that all the changes in the average properties of the proteins are due to the properties of these newly formed proteins. If so, the actual properties of these new proteins would differ from those of the older proteins to a more marked degree than is indicated by the curves, since these represent averages for both old and new proteins. But the possibility of changes in the old proteins is not excluded. The increase in quantity of proteins on exposure to room temperature is in agreement with the often reported hydrolysis of proteins to products of low molecular weight, at low temperatures (**7**). It may also explain why a change in average molecular weight sometimes occurs. This may possibly depend on the stage of resynthesis of the proteins.

The remarkable stability of the average molecular weight is shown in table II.

Discussion

It is, of course, impossible to decide from the above results whether they apply to the proteins as they occur in protoplasm, or whether the methods used markedly alter the properties of the proteins. Cell physiologists have concluded from their observations of living cells that the protoplasm of growing cells is more highly hydrated than that of resting cells (**3**). The fact that the results reported in this paper agree with this conclusion supports the belief that the isolated proteins retain, at least in part, the properties they possessed while in the protoplasm. The low average molecular weight obtained is perhaps against this view. Indeed, the most remarkable result was the relative stability of the average molecular weight, even when new proteins were being synthesized in considerable quantity. It is obvious, however, that some breakdown of the protoplasmic structure must occur during extraction—even if only at the "haftpunkte" described by FREY-WYSSLING (**3**). This would not necessarily alter the properties of the individual proteins.

The opposing tendencies of the dormant (internal) as compared with the active (external) tissues are interesting. It is usually stated that increased hydration of proteins occurs at low temperatures (**7**), yet this has always run counter to the above observations of cell physiologists that hydration increases during growth. The results reported here indicate that these apparently opposite concepts are both tenable, depending on the state of activity of the cells. However, the results reported are too few to permit any generalizations. But the fact that it is possible to detect differences in the properties of the proteins associated with differences in physiological state indicates that this method may prove a valuable one for investigating many problems.

Summary

1. A method is described for extracting protoplasmic proteins in the undenatured state from potato tubers and for determining their average molecular weight and hydration from osmotic pressure curves.

2. The average molecular weight of the protoplasmic proteins obtained from tubers stored at 0° C. was about 40,000–50,000 grams.

3. The protoplasmic proteins from the internal, dormant tissues were relatively stable in osmotic behavior. On transfer of the tubers from 0° to 25° C. for 6–7 days, the average molecular weight remained constant, and in most cases the hydration decreased slightly.

4. The protoplasmic proteins from the external tissues which were allowed to form new periderm proved more changeable in osmotic behavior. On transfer from 0° to 25° C. either an increase in hydration or a decrease in average molecular weight occurred.

5. Proteins were synthesized in considerable quantity when the tubers were transferred from 0° to 25° C. for 6–7 days. This is in agreement with the numerous reports by other investigators of protein hydrolysis at low temperatures.

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EFFECT OF INHIBITORS ON THE RESPIRATION AND STORAGE OF COTTONSEED

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(WITH THREE FIGURES)

Introduction

One of the earliest attempts to apply chemical treatment as a means of inhibiting the heating and lipolysis of cottonseed during storage was made by Barrow (**4**), who used sodium chloride as the effective chemical agent. The application of 5 per cent. of sodium chloride to moist cottonseed had a twofold effect. First, as a result of the high salt concentration outside the seeds, considerable moisture was withdrawn from within. Secondly, small quantities of the salt which diffused into the seed inhibited the biological processes responsible for heating and lipolysis. Malowan (**17**) found that sodium chloride likewise decreased the rate of evolution of carbon dioxide from moist cottonseed and that alcohol, acetic acid, sulphuric acid, and formalin inhibited respiration. He found that certain disinfectants, such as copper sulphate and mercuric chloride, had no effect on the rate of respiration and concluded, therefore, that the carbon dioxide evolution observed under his experimental conditions was caused not by the action of micro-organisms but rather by the action of enzyme systems operating in the seed.

Two of the present authors have reported previously (**13**) that ammonia and hydrochloric acid vapors inhibit lipolysis of the oil in cottonseed. Subsequent experiments indicated that ammonia treatment also effects the spectrum of extracted cottonseed oil and the heating of seed stored in bulk (**1**). This treatment of cottonseed with ammonia vapors, although potentially adaptable to commercial practice, was shown in mill-scale tests to be unfeasible as proposed (**2**).

In the present investigation the effect of ammonia upon a number of properties of both mature and immature cottonseed was measured in order to form a basis for a better understanding of the mechanism of inhibitor action. Since it was also of interest to determine whether the surface sterilization of cottonseed would seriously inhibit respiration and lipolysis or affect the color of the seed oil, a number of chemical agents known to act as inhibitors of microbial growth were also tested.

Materials and methods

The inhibitors used were ammonia, butyl maleimide, 2′-methyl-l-maleanil, Nacconol NR, Emulsol 607, and Emulsol 607M.

Both butyl maleimide and 2′-methyl-l-maleanil are compounds developed for use as disinfectants (**11**). The former, a liquid at room temperature,

is able to disinfect pointed steel strips inoculated with *Staphylococcus aureus* when the inoculated strips are suspended above the compound in a closed space for a period of 2 days. Spores of an Aspergillus species, when placed on glass slides and exposed to vapors of butyl maleimide in a closed space for 6 days at 50° C. or for 7 days at 25° C., do not germinate. Somewhat related in structure and properties to butyl maleimide, but less effective as a fumigant, is 2′-methyl-l-maleanil, which is a solid at room temperature.

Nacconol NR is the proprietary name for an alkyl aryl sodium sulfonate preparation which contains 36 per cent. organic matter, and possesses wetting and dispersing properties (**7**). Methods for preparing compounds of this type and descriptions of their chemical composition and structure are given in the patent literature (**8, 9, 10**). In addition to its detergent properties, Nacconal NR has germicidal properties, inasmuch as 1 : 500 dilution of the compound will destroy cultures of *Staphylococcus aureus* in 72 hours at 37° C. Nacconol NR is effective also in controlling fungus growth.

Emulsol 607, another type of detergent having germicidal properties (**6**), is described as the lauric acid ester of colamino formylmethyl pyridinium chloride. It will destroy cultures of *Staphylococcus aureus* in 10 minutes in a dilution of 1 : 20,000, and cultures of *Eberthella typhosa* in the same length of time in a dilution of 1 : 15,000. An indication of its fungicidal activity is given by the fact that it kills the spores of *Trichophyton interdigitale* and of *T. rosaceum* in a dilution of 1 : 20,000.

Emulsol 607M differs from Emulsol 607 in that it is derived from myristic acid and is a more effective bactericidal and fungicidal agent than Emulsol 607. Emulsol 607M was available and was used in the anhydrous form, whereas the Emulsol 607 was used in the form of a 10 per cent. aqueous solution.

Three varieties of cottonseed were used. Two of these, the "Delfos" and "Coker's 200-strain-1" were the same as used in experiments reported in a companion paper (**14**). The "Delfos" variety was grown in Stoneville, Mississippi in 1942, was fully matured when harvested, and was designated as the 100 series in the paper mentioned. The sample of seeds of the "Coker's 200-strain-1" variety was from bolls which had been harvested before they had properly matured and was previously designated as lot 204. This sample was grown in 1942, in Clemson, South Carolina. The third variety was "Cleve Wilt" grown in Clemson, during the 1943 season. It was fully matured when harvested and is designated as lot 405. Before being used in the inhibition experiments, the seeds were conditioned to a moisture content between 14 and 15 per cent. in the manner described in other papers (**13, 14**).

Ammonia was introduced by diffusing the gas through the seeds in a closed container for 1 hour. The seeds were then kept in the container (24 hours) to permit them to come to equilibrium with the excess of ammonia that had been adsorbed by the fuzz on the seed. At the end of this period, the pH of the seeds was approximately 8. In some of the experiments

reported, similar supplementary treatments with ammonia were given the seed during the course of the storage period.

Butyl maleimide was introduced by exposing 5 pounds of cottonseed for 1 week to the vapors emanating from 20 ml. of this substance. Exposure was accomplished by placing the seeds and the butyl maleimide in a desiccator which was then evacuated and sealed. At the end of the exposure period, the seeds were removed and placed in an air-tight container.

Inasmuch as Nacconol NR, 2′-methyl-l-maleanil, and Emulsol 607M were solids, treatment with these substances was accomplished by thoroughly mixing the seeds with the powdered material in the following proportions: One hundred grams of Nacconal NR with 5 pounds of seed, 20 gm. of 2′-methyl-l-maleanil with 5 pounds of seed; and 10 gm. of Emulsol 607M with 3 pounds of seed. Treatment with Emulsol 607 was accomplished by spraying 5 pounds of the seeds with 25 ml. of the solution in the form of a fine mist.

The treated seeds were stored in air-tight bottles at room temperature. Samples were removed periodically and examined. The analytical methods used for the determination of respiration intensity, lipolysis rate, pH, and spectrum of the seed oil were the same as described in other papers (**1, 3, 13, 14**).

Effect of inhibitor on respiration

In a series of respiration experiments conducted on the "Delfos" variety (100 series), all the chemical agents mentioned above, except Emulsol 607M, were used.

In its effect on the pattern of respiration, ammonia differed from all of the other chemical agents tested. This is evident from the representative respiration patterns of seeds of approximately the same moisture content which are shown in figure 1. The ammonia-treated seeds exhibited a relatively high initial respiration intensity followed in the intermediate portion of the storage period by low respiration, which began to rise again after 200 days' storage. The seeds which were treated with Nacconol NR, however, exhibited a maximum in respiration intensity during the storage period. Similar respiration patterns were given by the seeds treated with Emulsol 607, butyl maleimide, and 2′-methyl-l-maleanil. Such behavior is typical of most of the respiration experiments reported in a companion paper (**14**) (cf. figs. 1, 2, and 3).

The relationship between the ammonia concentration (pH) and the respiration intensity is apparent from an examination of table I, in which are given pH values for the ammonia-treated samples during the storage period. Sample 1 (average pH, 8.03) reached a pH of 8 at the end of the initial treatment and was maintained at or above that level for 3 months. This was in part due to the fact that additional ammonia treatments were given during the storage interval, as indicated in the table. After 150 days' storage, the pH of the seeds was slightly below 8, and from then on it dropped steadily to a final value of 7.75. Sample 2 (average pH, 7.91)

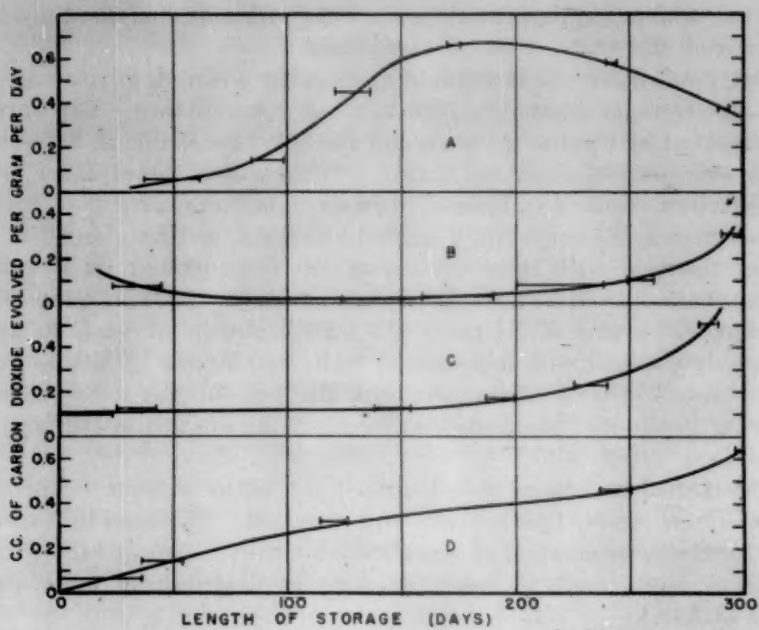

FIG. 1. Effect of inhibitors on the respiration pattern of "Delfos" variety cottonseed (100 series).

Curve	*Inhibitor*	*Moisture content, per cent.*
A	Nacconol NR	14.38
B	Ammonia (average pH of 8.03)	14.86
C	Ammonia (average pH of 7.91)	14.76
D	None	14.60

attained a slightly lower pH after the initial treatment than did sample 1, was maintained briefly at a pH above 8, and then was allowed to drop again to lower pH values. The initial stimulation of respiration indicated in

TABLE I

VARIATION OF pH IN STORED AMMONIA-TREATED COTTONSEED

LENGTH OF STORAGE	SAMPLE 1	SAMPLE 2
days	*pH*	*pH*
0	8.00	.....
4	8.05	7.80
25	8.10*	7.85*
32	8.00	7.77
53	8.23*	8.09*
71	8.23*	8.16*
90	8.17	8.12
147	7.94	7.83
218	7.88	7.77
326	7.75	7.79
Average	8.03	7.91

* These determinations were made following supplementary ammonia treatments.

curve B, figure 1, coincides with the initial heating observed following ammonia treatment (1). Such an initial stimulation of heating also occurred in mill-scale experiments involving the use of ammonia to improve the storage properties of cottonseed (2).

The respiratory patterns of the seeds treated with the inhibitors were integrated in the manner described (14) and their average respiratory intensities (RI) were determined. These values are compared in figure 2

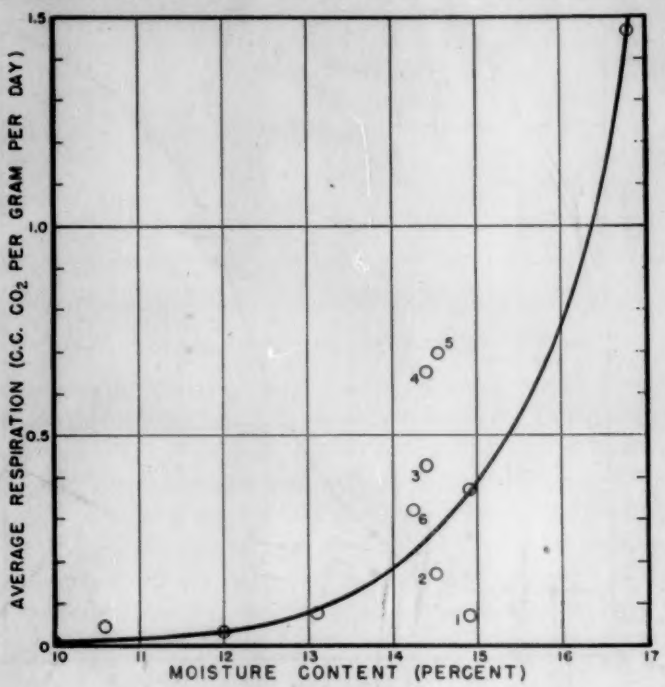

FIG. 2. Effect of inhibitors on the respiratory intensity of "Delfos" variety cottonseed (100 series), the curve representing the respiration of cottonseed in the absence of inhibitors.

1. Ammonia (average pH of 8.03)
2. Ammonia (average pH of 7.91)
3. Nacconol NR
4. Butyl maleimide
5. Emulsol 607
6. 2′ methyl-l-maleanil

with those attained on untreated seeds. The curve for untreated seeds shows the effect of moisture content on the respiratory intensity of "Delfos" variety seeds (100 series) as given in figure 4 of (14). It is interesting to note that only treatment with ammonia produced a lower-than-normal average respiratory intensity. The other treatments resulted in an overall stimulation of respiration, regardless of the form in which the fungicide or bactericide was applied.

Effect of inhibitors on lipolysis rate

Free fatty acid determinations were made on the same lots of seeds used for the respiration measurements, and the lipolysis rate constants (**13**) were calculated. These are compared in figure 3 with those obtained on normal untreated seeds of the same series. The curve is that for the "Delfos" variety cottonseed (100 series) which was given in figure 1 of a companion paper (**15**).

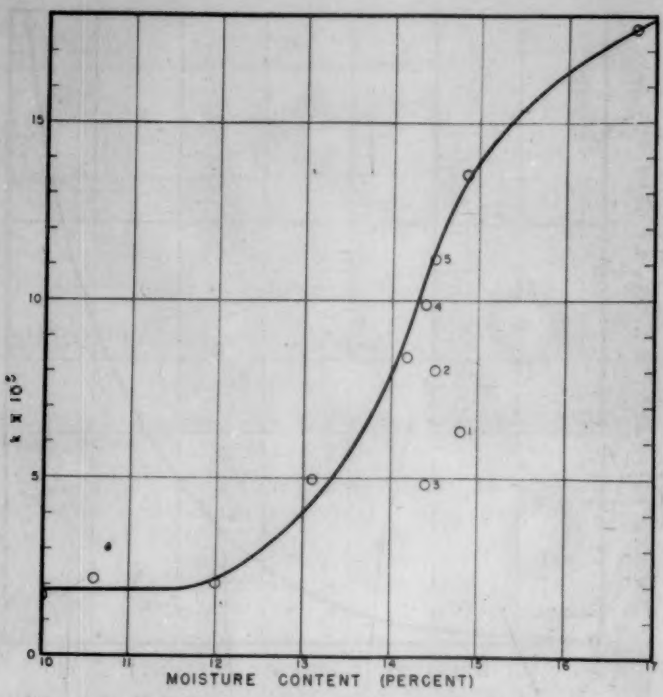

FIG. 3. Effect of inhibitors on lipolysis rate constant, k, of "Delfos" variety cottonseed (100 series), the curve representing lipolysis of cottonseed in the absence of inhibitors.

1. Ammonia (average pH of 8.03)
2. Ammonia (average pH of 7.91)
3. Nacconol NR
4. Butyl maleimide
5. Emulsol 607

There was no evidence that lipolytic rate was increased by any of the treatments. Emulsol 607 and butyl maleimide had no effect whatsoever. Ammonia had an inhibitory effect similar to that reported in a previous publication (**13**). Nacconol NR, and to a much lesser extent 2′-methyl-l-maleanil,[1] also reduced the rate of lipolysis.

[1] The curve representing lipolysis as a function of length of storage for the 2′-methyl-l-maleanil-treated seeds was not of the usual autocatalytic type, and, therefore, did not lend itself to the same type of kinetic analysis as did the curves for other normal or treated seeds. Nevertheless, a qualitative examination of data from storage of the seeds

Comparison of effects of inhibitor on respiration and lipolysis

Effects of the various treatments on respiration and lipolysis are compared in table II. Ammonia treatment produced consistent changes with

TABLE II

EFFECT OF VARIOUS CHEMICAL AGENTS ON THE RESPIRATION INTENSITY AND LIPOLYSIS RATE CONSTANT OF COTTONSEED

CHEMICAL AGENT	RESPIRATION	LIPOLYSIS
	% of normal	*% of normal*
Ammonia (average pH 8.03)	83	52
Ammonia (average pH 7.91)	38	29
Nacconol NR	169	55
Butyl maleimide	261	100
Emulsol 607	185	100
2′-Methyl-l-maleanil	145	Inhibited

respect to both processes, whereas the other treatments uniformly stimulated respiration, but either inhibited or had no effect on lipolytic activity.

Effect of ammonia concentration on lipolysis

A comparison of the results of the two ammonia treatments indicates that the extent of inhibition of lipolysis is a function of the concentration of the ammonia. This fact is clearly demonstrated in the following series of experiments conducted on cottonseed of the "Cleve Wilt" variety. Three-pound lots of seeds were conditioned to a moisture content of approximately 14.2 per cent. and set aside in air-tight bottles. One portion was used as the untreated control. A second portion was subjected to continuous ammonia treatment by placing in the bottle a small beaker full of ammonium carbonate which slowly decomposed and provided a constant supply of ammonia. A third portion was given only one initial ammonia treatment. A fourth portion was given an initial ammonia treatment, and then stored in an air-tight bottle for 30 days. At the end of this period, the ammonia was removed by withdrawing the seed samples from the bottle and spreading them in a current of air until the excess ammonia was dissipated. The seeds were then replaced in the original bottle and stored together with the other three lots.

The results of this experiment, together with those of certain other experiments to be described later, are shown in table III. The free fatty acid content of two of the ammonia-treated samples remained essentially unchanged for the first 100 days of storage. The third ammonia-treated sample (Lot 405eCr) showed a slight increase in free fatty acids (0.30%) during that period. Marked differences among the samples began to develop during the remaining 230 days of storage. The values of the lipolysis

treated with 2′-methyl-l-maleanil indicated that the rate of free fatty acid formation was definitely inhibited. At the end of 340 days' storage, the sample treated with 2′-methyl-l-maleanil developed 12.5 per cent. free fatty acids, as compared to 17.5 per cent. developed by an untreated control of the same moisture content.

rate constant, k, given in table III represent the overall rates for the entire storage period. It is clear that to realize the maximum effect of inhibition by treatment with ammonia it is necessary to continuously subject the seeds to the action of ammonia vapors.

Nacconol NR was previously found to be the only one of the agents tested which inhibited the lipolytic activity of the cottonseed to a very marked extent. Accordingly, another experiment (405eNv) was planned to determine whether the biological activity of Nacconol NR was due to direct contact with the seeds or to the action of vapors emanating from this material. Three pounds of cottonseed, previously conditioned to 14 per cent. moisture content, were stored in a desiccator above a dish containing 60 gm. of Nacconol NR. In order to compare the activity of solid Emulsol

TABLE III

EFFECT OF INHIBITORS ON THE LIPOLYSIS RATE OF "CLEVE-WILT" VARIETY COTTONSEED

EXPERIMENT	INHIBITOR	MOISTURE CONTENT	FREE FATTY ACID CONTENT			LIPOLYSIS RATE CONSTANT, K	INHIBITION
			ORIGINAL	100 DAYS	230 DAYS		
		%	%	%	%	*reciprocal of days*	%
405 e	None	14.5	0.83	7.14	40.30	20.4×10^{-5}	.....
405 eCC	Continuous ammonia	14.7	0.83	0.65	1.78	3.4 "	83.3
405 eC	Single ammonia treatment	14.3	0.83	0.85	7.14	9.6 "	52.9
405 eCr	Excess ammonia removed	14.2	0.83	1.13	28.35	16.7 "	17.9
405 eNv	Nacconol NR vapors	14.0	0.83	1.46	2.75	5.5 "	73.0
405 eE	Emulsol 607M	14.2	0.83	7.21	39.99	20.4 "	None

607M with the previously determined action of an aqueous solution of Emulsol 607, a second experiment (405eE) was conducted by mixing and storing 3 pounds of seed and 10 gm. of Emulsol 607M. From the results given in table III, it is clear that vapors emanating from Nacconol NR have powerful inhibiting properties. On the other hand, Emulsol 607M, a more powerful germicide than Emulsol 607, exhibits no inhibiting effect on the lipolysis rate.

The discovery that vapors from Nacconol NR preparations inhibit the formation of free fatty acids in cottonseed may be of great practical significance to the cottonseed industry. Preliminary mill-scale experiments on the use of this material to improve the storage properties of cottonseed and flaxseed have indicated that improvement may, in fact, be achieved (**2**). Further investigations designed to determine the nature and mode of action of the biologically active vapors are in progress.

Effect of inhibitors on the spectrum of extracted oils

The observation, reported in a previous publication (**1**), that ammonia treatment of cottonseed reduces the color of the solvent-extracted oils was

confirmed and extended in the present investigation. It was found, however, that the inhibitors other than ammonia which were tested did not affect the spectrum of the oil. In table IV are shown the results of the effects of various inhibitors on the light absorption of the solvent-extracted cottonseed oils at 560 mμ, the wavelength of maximum light absorption of gossypurpurin (**5**). It can be seen that the concentration of ammonia influences the rate at which the absorption at 560 mμ is reduced. A similar effect was observed when the light absorption of the oil from ammonia-

TABLE IV

THE RELATIVE LIGHT ABSORPTION COEFFICIENT AT 560 mμ OF THE OILS FROM "DELFOS" VARIETY COTTONSEED (100 SERIES) AS AFFECTED BY VARIOUS INHIBITORS

INHIBITOR	LENGTH OF STORAGE	LOG I_o/I*	INHIBITOR	LENGTH OF STORAGE	LOG I_o/I
	days			*days*	
None	29	0.018	Ammonia (ave. pH of 8.03)	32	0.012
	120	0.017		90	0.006
	156	0.011		147	0.004
	337	0.028		326	0.007
Ammonia (ave. pH of 7.91)	32	0.014	Nacconol NR	29	0.018
	90	0.009		71	0.018
	147	0.007		157	0.012
	326	0.006		337	0.038
Emulsol 607	29	0.018	Butyl maleimide	29	0.018
	71	0.018		71	0.021
	157	0.012		158	0.018
	337	0.013		337	0.017
2′-Methyl-1-maleanil	29	0.018			
	71	0.017			
	158	0.012			
	337	0.017			

* All absorption data are calculated for solutions in carbon tetrachloride containing one ml. of oil in a total of 50 ml. of solution. The absorption cell length was 13 mm.

treated seeds at 360 mμ, the wavelength of maximum light absorption of gossypol, was compared to that of the oil from the untreated control.

Effect of ammonia on immature cottonseed

In the course of previous work (**14, 15**), it was noted that an immature sample of seeds ("Coker's 200, strain 1") behaved entirely differently, with respect to both lipolysis rate pattern and respiration intensity, from all of the other samples investigated. The present experiments show that this difference in behavior is also reflected in the reaction of the seeds to ammonia treatment.

When immature seeds were treated with ammonia to raise the pH of the sample to 8.3, the overall respiratory intensity was greatly reduced, in spite of a marked stimulation of respiration immediately following exposure of the seeds to ammonia vapors. Thus, the overall respiratory intensity of untreated immature seeds (sample 204b) was 0.14 cc. of carbon dioxide

per gram per day, compared to 0.0027 cc. for comparable ammonia-treated seed (sample 204c). The moisture contents of the two lots were practically the same; *i.e.*, 12.5 per cent. for sample 204b and 12.1 per cent. for sample 204c. The decrease in respiratory intensity was much greater than that usually encountered when mature seeds were treated with ammonia. For example, mature ammonia-treated seeds ("Delfos" variety) which were kept at an average pH of 8.03 had a respiratory intensity of 0.07 cc. of carbon dioxide per gram per day compared to a value of 0.37 for the untreated control lot of the same moisture content. This represents an inhibition of 81 per cent. as compared to an inhibition of 98 per cent. occasioned by similar treatment of immature seeds. In addition to the quantitative differences in respiration intensity between the immature and normal seeds, the respiratory quotient of the ammonia-treated immature seeds (sample 204c) was found to be 0.88 as compared to 0.95 for untreated immature seed (sample 204b). On the other hand, ammonia was found to exert no effect on the respiratory quotient of mature seeds.

TABLE V

CHANGES IN LIGHT ABSORPTION AT 360 mμ AND 560 mμ OF THE OILS EXTRACTED FROM IMMATURE SEEDS (SAMPLE 204) DURING STORAGE

SAMPLE								
204A			204B			204C		
TREATMENT								
NONE			MOISTURE CONTENT REDUCED			AMMONIA		
MOISTURE CONTENT								
13.7 PER CENT.			12.5 PER CENT.			12.1 PER CENT.		
LENGTH OF STORAGE	LOG I_o/I 360 mμ	LOG I_o/I 560 mμ	LENGTH OF STORAGE	LOG I_o/I 360 mμ	LOG I_o/I 560 mμ	LENGTH OF STORAGE	LOG I_o/I 360 mμ	LOG I_o/I 560 mμ
days			*days*			*days*		
0	12.60	0.030	0	8.24	0.041	0	3.19	0.078
41	6.74	0.034	31	4.42	0.031	31	2.59	0.062
69	4.72	0.036	67	3.95	0.033	53	2.16	0.062
112	3.51	0.041	110	2.94	0.048	87	1.96	0.077
142	3.44	0.051	140	3.78	0.048	138	0.74	0.079
212	4.46	0.070	210	6.43	0.074			

Whereas the respiration of immature cottonseed was inhibited by ammonia, this treatment had the opposite effect on the rate of lipolysis. As was shown in figure 5 of a companion paper (**15**), the lipolysis patterns of immature and mature seeds differed fundamentally; the results obtained on immature seeds could not, therefore, be subjected to the same type of mathematical analysis as was used in the case of the results obtained on mature seeds. Both the ammonia-treated and untreated immature seeds gave a similar type of lipolysis pattern. Ammonia stimulated lipolysis of immature seeds, whereas it invariably inhibited lipolysis of the mature seeds. After 100 days of storage, the untreated immature seeds with a 12.5 per

cent. moisture content developed 16 per cent. free fatty acids; but after the same period, the ammonia-treated immature seeds with a 12.1 per cent. moisture content developed 24.5 per cent. free fatty acids.

The effects of ammonia treatment on the absorption spectra of oils extracted from immature seeds and on those extracted from mature seeds were also profoundly different. In table V are given the light absorptions at 360 mμ and 560 mμ of the oils from the three lots of immature seeds as a function of length of storage. In table VI is given a summary of all of the types of spectral changes encountered. The untreated immature seeds exhibited at 560 mμ a pattern of change which was not of the same nature found for the mature seeds. Thus, except for an initial drop, the oil from sample 204b exhibited a steady rise in light absorption as the length of the storage period increased. Sample 204a exhibited a steady rise in light absorption from the very beginning of the storage period. On the other hand, the ammonia-treated immature seeds (sample 204c) had an initially higher light absorption in this region, and this absorption did not vary throughout the storage period. The ammonia treatment of mature seeds has been previously shown to lower gradually the light absorption of the extracted oil in the same region (**15**).

TABLE VI

EFFECT OF STORAGE ON THE PATTERN OF CHANGES IN LIGHT ABSORPTION OF THE OILS EXTRACTED FROM UNTREATED AND AMMONIA-TREATED, MATURE AND IMMATURE COTTONSEED

VARIETY	TYPE	TREATMENT	PATTERN OF CHANGES AT 360 mμ	PATTERN OF CHANGES AT 560 mμ
"Delfos"	Mature	None	Reversal of change	Reversal of change
		Ammonia	Gradual drop	Gradual drop
"Coker's-200 strain 1"	Immature	None	Reversal of change	Steady rise
		Ammonia	Gradual drop	No change

With respect to absorption in the 360 mμ region, the two untreated immature seed samples exhibited a reversal of change, whereas the ammonia-treated immature sample showed the steady decrease which is characteristic of treated mature seeds. Thus ammonia treatment has a similar effect on the spectra of the oils of both mature and immature seeds in the 360 mμ region.

The data in table VI confirm the observation made by BOATNER (**5**) that the light absorptions at 360 mμ and at 560 mμ are not due to the same pigment systems. In the mature seeds, these pigments are affected similarly by both storage and ammonia treatment. In immature seeds, however, these two pigment systems behave independently of each other.

Discussion

The initial stimulation of respiration, observed when cottonseed is treated with ammonia, is common to many types of reactions involving poisons and

drugs. IRVING (**12**) observed that the respiration of leaves was initially stimulated by exposure to chloroform vapors; when the concentration of chloroform was raised sufficiently, this initial stimulation was rapidly followed by an inhibition of respiration. The initially higher respiration which resulted from the treatment of seeds with ammonia was of such a short duration that the overall effect of this treatment was a decrease in the respiratory intensity of the seeds. It is conceivable that had the concentration of ammonia in the seeds been kept sufficiently low throughout the storage period, the stimulation would have been maintained throughout, and the net effect would have been an increase in respiratory intensity over that of the normal untreated seeds.

An increase in respiratory intensity is what actually occurred in the seeds treated with Nacconol NR, Emulsol 607, butyl maleimide, and 2′-methyl-l-maleanil. In every case, there was a stimulation of respiration which persisted throughout the storage period and resulted in a higher than normal value for respiratory intensity. In large-scale storage experiments, however, it has been possible to increase the concentration of inhibitor (Nacconol NR vapors) to such an extent that the initial stimulation of biological activity is soon replaced by marked inhibition (**2**).

It is significant that the inhibitions of respiration and of lipolysis are not strictly parallel phenomena. That it is possible to inhibit one activity and stimulate the other in the same lot of seeds was shown most clearly by the effect of the Nacconol NR treatment. Thus, it would seem that the processes of respiration and lipolysis are not functionally related in resting seeds; one system may be inhibited under conditions that will stimulate the other. The system involved in lipolysis is apparently more easily inhibited than is that involved in respiration; this is evident in the results obtained on mature seeds where it can be seen there are a number of chemical treatments which inhibit lipolysis but stimulate respiration.

The lack of parallelism between respiration and lipolysis suggests a possible method for distinguishing between deterioration caused by the bacteria and molds that are associated with the seeds and that resulting from the operation of the enzyme systems of the seed themselves. It has been tacitly assumed in many investigations of the deterioration of seeds during storage, that the micro-organisms associated with the seed are the primary cause of deterioration. Thus RAMSTAD and GEDDES (**19**) have suggested that most of the heating that takes place in moist soybeans is due to the action of micro-organisms. Similarly, deterioration in flaxseed has been attributed to microbial activity (**16, 20**). Even though surface sterilization of the seeds rarely affected their rate of respiration or heating, results obtained by use of such procedures could not be offered as clear-cut evidence that the respiration was independent of bacterial action, because surface sterilization obviously could have no effect on the micro-organisms present in the interior of the seeds. Any attempt to achieve sterilization within the seeds would, of course, yield inconclusive results because biological systems

of the seeds would also be affected by such a sterilization procedure. Yet, if micro-organisms were the sole cause of respiration, heating, and lipolysis obtained in stored seed, it would be expected that the inhibition of the growth of such organisms would affect all of these processes in a similar manner. On the other hand, if most of the deteriorative action were due to systems within the seeds themselves, it is conceivable that certain biological functions would be more susceptible to inhibition than others. Therefore, when these criteria are applied to the behavior of cottonseed and are considered in conjunction with the evidence presented in this and the preceding papers, it becomes abundantly clear that the bulk of the deteriorative processes in stored cottonseed, such as respiration, heating, and lipolysis, are due to the activity of seed enzyme systems.

The evidence upon which this conclusion is based may be summarized as follows:

1. Surface sterilization has no effect on the respiration of cottonseed. Despite the variety of the fungicides and bactericides used, none has been found to inhibit respiration. These results confirm those of MALOWAN (**17**) who showed that solutions of mercuric chloride or copper sulfate had no inhibitory effect on the heating of cottonseed. Obviously, therefore, surface growth of micro-organisms does not materially contribute to the heating or respiration of the seeds.

2. Nacconol NR has a differential effect on the respiration and lipolysis of cottonseed; under the experimental conditions that were generally used, respiration was stimulated, whereas lipolysis was markedly inhibited. With the exception of the ammonia treatment, the chemical treatments applied stimulated respiration, but they had no such effect on lipolysis.

3. Under certain conditions, it was possible to effect by chemical treatment an inhibition of respiration but a stimulation of lipolysis. Thus, the treatment of immature seeds with ammonia resulted in greatly reduced respiration, but increased lipolysis.

4. Every aspect of the behavior of immature seeds (the 204 series) differs from that of mature seeds; respiration is much higher than normal; the lipolysis pattern is different; spectral changes and the reaction to inhibitors differ markedly from those of normal seed. It is inconceivable that the microbial population of immature cottonseed should differ so greatly from that of mature cottonseed as to change both the degree and pattern of respiration, lipolysis, and spectral changes.

It is possible to conclude, therefore, that under the experimental conditions of temperature and moisture reported in this series of publications, the major portion of the biological activity observed results from the activity of seed enzyme systems. The initial stages of deterioration including the initial heating caused by respiration (**1**, **2**, **18**), which occur in cottonseed during commercial bulk storage, are almost certainly a result of seed enzymatic activity. Unquestionably, the microbial growth which takes place during storage may reach such proportions as to contribute to

the apparent biological activity of the seeds. But this microbial effect is secondary in nature and is evident only after prolonged storage.

It follows from these conclusions that, with the exception of seeds having excessively high moisture content, any attempt to reduce the biological activity of stored cottonseed must concern itself first with the inhibition of the seed enzyme systems.

Summary

1. Treatment of cottonseed with ammonia was found to inhibit the respiration and lipolysis of mature seeds and reduce the light absorption of the extracted oil at 360 mμ.

2. Similar treatment of immature cottonseed was found to inhibit respiration, but to stimulate lipolysis. Light absorption of the extracted oil at 560 mμ was increased, whereas the oil from ammonia-treated mature seeds exhibited decreased absorption at this wave length.

3. The vapors of Nacconol NR were found to inhibit lipolysis in cottonseed under conditions where there was a stimulation of respiration. Treatment of cottonseed with 2′-methyl-l-maleanil yielded similar results.

4. Fungicides and germicides such as Emulsol 607M, Emulsol 607, and butyl-maleimide had no effect on the lipolysis rate of stored cottonseed; the last two substances stimulated respiration.

5. Evidence has been presented to demonstrate that most of the deterioration which occurs in stored cottonseed is due to the action of enzymes in the seeds rather than to microbial activity.

The authors wish to thank G. W. Irving, Jr. and F. G. Dollear for many helpful suggestions. The samples of Nacconol NR, butyl maleimide, and 2′-methyl-l-maleanil were kindly furnished by Lawrence H. Fleet, of the National Aniline Division of the Allied Chemical and Dye Corporation, and the samples of Emulsol 607 and Emulsol 607M were furnished through the courtesy of Albert K. Epstein of the Emulsol Corporation.

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EFFECT OF 2,4–DICHLOROPHENOXY ACETIC ACID ON THE RIPENING OF BARTLETT PEARS[1]

ELMER HANSEN

(WITH ONE FIGURE)

It has long been known that ethylene and certain other unsaturated hydrocarbon gases have the property of stimulating the ripening of various fruits. Recent data indicate that similar effects can be produced by several of the synthetic plant-growth hormones. Thus, the maturation and ripening of certain varieties of apples and pears have been observed to be directly influenced by naphthalene acetic acid when used as a spray to retard harvest-drop (**1, 2**). MITCHELL and MARTH (**6**) found that the time required for the ripening of green bananas and freshly-harvested apples and pears was reduced by treatment with 2,4-dichlorophenoxy acetic acid. These treatments were applied to fruits in the pre-climacteric stage when the quantity of ethylene metabolized by the tissues is known to be extremely small (**4, 5**). No data are available to indicate their effect in the presence of physiologically active quantities of ethylene, such as occur in post-mature and storage fruits. In the present study, the comparative effects of 2,4-dichlorophenoxy acetic acid on the respiration and ripening of Bartlett pears, which vary in stage of maturity and capacity for ethylene production, are considered.

Materials and methods

Two collections of pears were made from a single tree located at the Experiment Station orchard near Corvallis. The first sample was picked on August 14 and represented fruit in an immature stage of development. The second sample was collected two weeks later when the fruit was at the optimum stage of maturity for commercial use. A portion of this collection was stored at 31° F. for five weeks prior to treatment.

The method used in treating pears with 2,4-dichlorophenoxy acetic acid consisted of immersing the fruit in an aqueous solution containing 1000 p.p.m. of the reagent and one per cent. Carbowax. In some experiments other dilutions were used as indicated later. The treated fruits were allowed to dry before transferring to the respiration chambers.

The rates of carbon dioxide and ethylene production were determined by the methods described previously (**3**).

Results and discussion

IMMATURE FRUIT

Immediately after picking, the pears were divided into four uniform lots, each consisting of 15 fruits. Lot 1 was treated with 2,4–D and then con-

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fined in a desiccator in order to retain the ethylene emanating from the fruit. Lot 2 was untreated but similarly confined during the course of the experi-

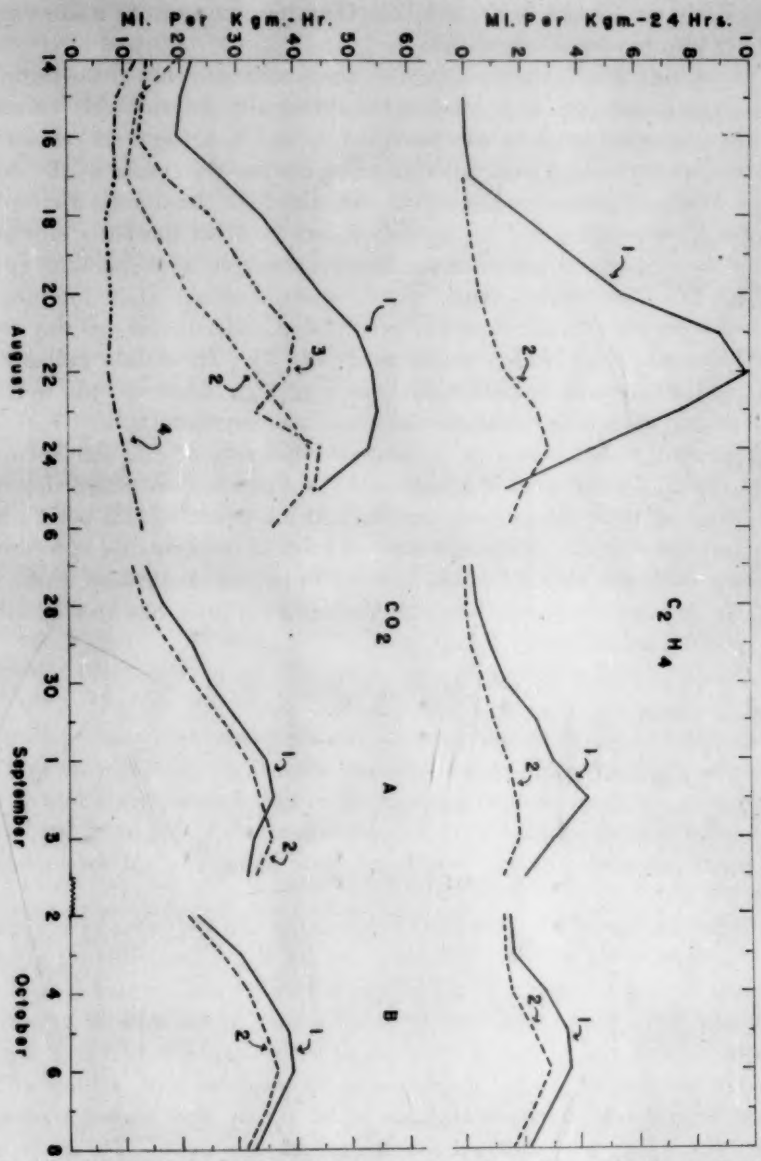

FIG. 1. Effect of 2,4-dichlorophenoxy acetic acid on the respiration and ethylene production of Bartlett pears. 1, treated-unaerated; 2, untreated-unaerated; 3, treated-aerated; 4, untreated-aerated. A, mature fruit ripened before storage; B, mature fruit ripened after 5 weeks' storage.

ment. Lot 3 was treated with the acid, then placed in a desiccator which was aerated with a constant flow of fresh air (20 liters per hour) in order

to remove ethylene from the storage atmosphere. Lot 4 was untreated and constantly aerated. The rates of carbon dioxide production were determined daily on all lots, while ethylene determinations were made only on fruit kept in the closed containers.

The amount of ethylene produced by the immature pears at the beginning of the experiment (fig. 1) was below the chemically determinable minimum, and when constant aeration was provided, as in Lot 4, the fruit remained in the preclimacteric stage and failed to ripen during the course of the experiment. When ethylene was allowed to accumulate in the storage atmosphere, as in Lot 2, respiratory activity increased and the fruit was fully ripe at the peak of the climacteric. Similar stimulation occurred in the samples treated with 2,4–D. The treated fruit, which was unaerated (Lot 1), however, attained a higher maximum rate of respiration and ripened one day sooner than the treated fruit, which was aerated (Lot 3). These data indicate that 2,4–D and ethylene in combination have a greater influence on respiration and ripening than either reagent has when used separately.

Treatment with 2,4–D greatly increased the rate of ethylene formation by the fruit. In the treated sample, ethylene production attained a maximum value of 10.06 ml. as compared to 2.84 ml. per kilogram per 24 hours in the untreated fruit. This represents a 3.6-fold increase due to treatment, and since there was only a 1.3-fold increase in rate of respiration in the same fruit, the production of ethylene and carbon dioxide do not appear to have been equally influenced by 2,4–D.

Mature fruit

The rates of ethylene and carbon dioxide production were determined on treated and untreated pears before and after five weeks' storage at 31° F. In addition, the rates of ripening were determined on separate lots of fruit which were treated with 2,4–D in concentrations of 10, 50, 100, 250, 500 and 1000 p.p.m. then kept in well-ventilated room maintained at a temperature of 65–70° F.

The initial rate of ethylene production in the mature pears before storage was less than 0.001 ml. per kilogram per 24 hours, and during ripening increased to 1.86 ml. and 4.31 ml. in the untreated and treated lots, respectively (fig. 1). There was very little difference in the rate of respiration between the two lots, although the peak of the climacteric occurred one day sooner in the treated fruit. As indicated by pressure tests, all lots of fruit treated with 2,4–D in concentrations of 50 p.p.m. and higher ripened in seven days, while the untreated lot ripened in nine days. The green color of the fruit treated with the acid in concentrations of 100 p.p.m. and higher failed to disappear uniformly, resulting in the development of a yellow-green, mottled appearance. This condition was not evident on fruit treated only with one per cent. Carbowax, nor in fruit treated with 2,4–D after five weeks of cold storage.

The pears which had been kept in cold storage for five weeks were still

firm and green and differed but little in outward appearance from freshly-harvested fruit. The data show, however, that the respiratory activity and the capacity for ethylene production were much greater than before storage, and it is evident that the climacteric was in progress at time of treatment. Both carbon dioxide and ethylene production tended to be slightly higher in the treated fruit, but the peak of the climacteric in both treated and untreated lots occurred on the same day. All lots of fruit, including the controls as well as those treated with varying concentrations of 2,4–D were fully ripe on the sixth day. According to these data, the time required for the ripening of Bartlett pears is unaffected by 2,4-dichlorophenoxy acetic acid when treatment is applied after the climacteric has been initiated.

Summary

Aqueous solutions of 2,4-dichlorophenoxy acetic acid in 1 per cent. Carbowax were applied to Bartlett pears at different stages of maturity and their effect on ripening, respiration and ethylene production determined.

Treatment of premature pears resulted in an increase in the rates of ripening, respiration, and ethylene production. The maximum values for carbon dioxide and ethylene production were 1.3 and 3.6 times greater, respectively, in the treated than in the untreated fruits. Ethylene and 2,4-dichlorophenoxy acetic acid in combination appeared to have a greater effect on ripening, respiration and ethylene production than either reagent used separately.

Mature pears treated shortly after harvest showed higher rates of respiration and ethylene production and ripened two days sooner than similar untreated fruit. The time required for the ripening of mature pears which had been stored at 31° F. for five weeks was not reduced by treatment with 2,4-dichlorophenoxy acetic acid. The rates of carbon dioxide and ethylene production, however, tended to be higher in the treated fruits.

The author is indebted to Dr. Paul C. Marth, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agriculture Research Administration, U. S. Department of Agriculture, Beltsville, Maryland, for furnishing the 2,4-dichlorophenoxy acetic acid solutions in Carbowax.

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Colloids, Their Properties and Applications.—A. G. WARD. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 133 pages. $1.75.

This small book presents a non-technical, introductory account of the physics and chemistry of colloids with many references to their applications in physics, engineering, geology, and biology. Though there is relatively little emphasis given to colloids in biology, the book is nevertheless of value to biologists because of the discussion on general characteristics of colloids which comprises the largest portion of the text. A brief bibliography lists the standard reference books on colloids and physical chemistry. The book will be of interest to students wishing to obtain a concise, non-mathematical overview of the colloidal state and common colloidal systems. The text is liberally illustrated, clear, and concise. A subject index permits rapid reference to specific topics.

Currents in Biochemical Research.—Edited by D. E. GREEN. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 486 pages. $5.00.

Thirty-one essays are presented in this volume outlining the present course of biochemical research as it relates to agriculture, medicine, and social problems. Each unit is written by a well-qualified specialist in a style and terminology devoid of abstruse technicalities. The book is in the nature of a concise digest aimed to acquaint scientists with trends, techniques, and needs in various phases of biochemical study. Each section carries a bibliography of the most important recent researches upon which the discussions are based. References do not include titles except for books cited. There is no general index to the volume itself.

Essays of special interest to plant scientists include: The Gene and Bio chemistry, Viruses, Photosynthesis, Nutrition of Plants, The Bacterial Cell, Enzymes, Enzymatic Carbon Dioxide Assimilation, Oxidation and Reductions, Isotope Techniques, and Plant Hormones. The book serves an important purpose in permitting a rapid acquaintance with an extremely broad area of research without the laborious effort of consulting the original literature of the many subjects discussed. The essays are lucid and accurate. The authors, editor, and publishers deserve commendation for their preparation of a most well-selected collection of biochemical essays which will be welcomed by chemists, physicists, and biologists as a means of maintaining contact with developments in extremely diversified fields of biochemical research.

Forest Soils and Forest Growth.—S. A. WILDE. Volume XVIII of A New Series of Plant Science Books, 1946. Chronica Botanica Co., Waltham 54, Massachusetts and G. E. Stechert and Co., 31 E. Tenth St., New York, N. Y. 241 pages. $5.00.

This book is something of a pioneer in its field. The author discusses soils from the standpoint of composition of forest stands and their effect upon individual trees, especially in relation to morphological pattern, growth rate, quality of wood, reproductive vigor, and resistance to adverse biotic and climatic conditions. Soils are viewed as carriers of definite floristic associations, as media for growth of nursery stock or forest plantations and as dynamic systems varying in the reaction to different forms of silvicultural treatment.

Several chapters are devoted to the genesis and genetical classification of soils in relation to silviculture followed by descriptions of the physical, chemical, and biotic properties of forest soils. There is an excellent chapter on the biological structure of forest cover and its relation to environment. Other chapters deal with soils in relation to forest management and to sound silvicultural practice. The book includes a very comprehensive bibliography as well as separate author and subject indexes. The author has performed an excellent service in correlating and interpreting the research of soil science, ecology, and physiology in relation to the growth and forestry procedures. The format of the book and numerous illustrations facilitate reading and comprehension. The personal background of the author has permitted him to correlate especially well the European and American researches in relation to forest soils.

Enzymes and Their Rôle in Wheat Technology.—Volume I, Monograph Series, edited by J. ANSEL ANDERSON. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 371 pages. $4.50.

This is the first of a series of monographs to be published by the American Association of Cereal Chemists primarily as a service to its members, but also to biologists in general. The introductory chapter, devoted to the general chemistry of enzymes, is followed by twin discussions of carbohydrase, protease, esterase, oxidase, and fermentive enzymes. The first of the two essays on each topic is a general discussion of existing knowledge of a particular group of enzymes, followed by a section on the technology of the same group, especially in relation to the use of cereals. Each section is written by a well-qualified expert and contains a very complete bibliography including subject titles as well as author and journal citations.

The text is abundantly illustrated and the format permits easy reading. Separate author and subject indexes facilitate rapid reference, adding materially to the usefulness of the book. The editor and authors have made an excellent choice of materials and integrated an extremely diversified mass of data into a logical and coherent account. The general discussions of particular groups of enzymes make this monographic review of interest to all biologists.

Annual Review of Biochemistry.—Volume XV, edited by JAMES MURRAY LUCK. Annual Reviews, Inc., Stanford University, California. 687 pages. $5.00.

Contained in this volume are 21 separate sections of which the following are of particular interest to plant scientists: Plant Carbohydrates by S. Peat; Growth Factors for Microorganisms by E. E. Snell; Photosynthesis by C. S. French; The Respiration of Plants by W. O. James; Biochemistry of Yeast by C. Neuberg; Bacterial Metabolism by H. A. Barker and M. Doudoroff; The Vitamins by R. A. Dutcher and N. B. Guerrant; Organic Insecticides by W. M. Hoskins and R. Craig; The Viruses by N. W. Pirie; Biological Oxidations and Reductions by K. A. C. Elliott; Non-oxidative Enzymes by A. M. Wynne; and The Chemistry of the Steroids by T. Reichstein and H. Reich. Each section carries a comprehensive bibliography. The volume has separate and very complete subject and author indexes.

Advances in Enzymology.—Volume VI, edited by F. F. NORD. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, N. Y. 563 pages. $6.50.

This volume is now ready and includes sections on the following subjects: Bacterial Amino Acid Decarboxylases by E. F. Gale; Enzyme Problems in Relation to Chemotherapy by M. G. Sevag; Biological Antagonisms between Structurally Related Compounds by D. W. Woolley; Adenosine-triphosphatase Properties of Myosin by V. A. Engelhardt; States of Altered Metabolism in Diseases of Muscle by C. L. Hoagland; Acetyl Phosphate by Fritz Lipmann; Microbial Assimilations by C. E. Clifton; Chemical Changes in Harvested Tobacco Leaf by W. G. Frankenburg; Action of the Amylases by R. H. Hopkins; Amylases of Wheat and Their Significance in Milling and Baking Technology by W. F. Geddes; and Tocopherol Interrelationships by K. C. D. Hickman and P. L. Harris.

In harmony with the policy of Advances in Enzymology, the foregoing critical reports cover borderline subjects between physiology, microbiology, chemistry and physical chemistry. Each section contains a complete bibliography. This volume (VI) carries separate cumulative subject and author indexes for volumes I to VI as well as the usual indexes to volume VI.

The Periodic Partial Failures of American Cottons in the Punjab: Their Causes and Remedies.—R. H. DASTUR. Indian Central Cotton Committee, Bombay. 144 pages. $2.00.

Dastur has assembled the major results on this topic as they have appeared in the Indian Journal of Agriculture Science and other periodicals. The importance to India of the replacement of short staple indigenous cottons by American varieties makes the publication an extremely useful reference. Discovery of the fact that cotton failure or Tirak disease was correlated either with light, sandy soils low in nitrogen, or with sub-soils of high alkalinity was not only of practical importance but resulted in perfection

of new methods of cotton planting, culture, and fertilization which largely overcome otherwise frequent crop failures.

The author traces the history of American cottons in India, procedures used in identification and experimental reproduction of physiological failures, as well as of various successful methods of avoiding losses. In addition to the value of the data to cotton growers, the book provides excellent examples of the application of purely scientific methods in isolation and identification of salient factors involved in complicated plant responses. The general method of attack and details of procedure are of interest to agronomists and physiologists in general in relation to problems connected with practical crop production. Data are well organized and clearly presented. A selected bibliography on cotton is included.

The Naturalists' Directory.—Current edition September, 1946, Salem, Massachusetts. $3.00 postpaid. The directory contains names, addresses, and special subjects of study of professional and amateur naturalists throughout the world. It has been published regularly for sixty years.

GENERAL INDEX[1]

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